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EXPERIMENTAL DATA FOR A REVISION OF THE NORTH AMERICAN WILD ROSES[1]

EILEEN WHITEHEAD ERLANSON

(WITH TWENTY FIGURES)

Experimental taxonomy

Within the past ten years the problem of species differentiation in several large plant genera has been attacked by a combination of ecological, genetical, and cytological work, with illuminating results. This method of approaching taxonomic problems was originally called genecology by TURESSON (**40**), a word to which there are some objections in English. BABCOCK has for many years been an enthusiastic exponent of what he designates as cyto-genetics (**2**), and has carefully analyzed the various types of heritable intraspecific differences in *Crepis* (**4**). The work of the late DR. H. M. HALL on transplant experiments has contributed valuable information on the evolution of species; this work was aptly called experimental taxonomy (**27**).

Naturally annual species gave good cyto-genetical data rapidly, and those on *Crepis*, *Nicotiana*, *Viola*, *Triticum*, *Antirrhinum*, *Oenothera*, and *Papaver* are already exhaustive. Many valuable contributions have been made which consist of careful cyto-genetical studies of a few species or species hybrids within a genus (*Phleum*, *Aquilegia*, *Salix*).

Among attempts to study perennial, fruticose, and woody genera,

[1] Paper from the Department of Botany of the University of Michigan, no. 437, representing work done under a National Research Fellowship in the Biological Sciences.

HURST's ambitious plan to make a thorough analysis of the genus *Rosa* has met with several reverses. Specific data on crosses have not been published, nor have the details of intraspecific variation. VAN ESELTINE and NEBEL have begun to attack the species problem in *Malus*, although they can hope to see only three or four generations of apples in their lifetime (**41**). Valuable results are to be expected from the work in progress at the laboratory of the Carnegie Institution at Stanford on *Pentstemon*, *Hemizonia*, *Zauschneria*, and *Potentilla* (**27**).

The roses of the North American continent present a problem which demands a new mode of approach. The method that has gradually been worked out is very similar to that expounded by HALL (**27**), the successive steps of which are: (1) Examination and analysis of herbarium specimens. (2) Field expeditions to observe and collect the living plants in their habitats. (3) Observation of the same living plants in garden culture that were studied in the field, and preparation of herbarium specimens from them. Further steps that have been added to HALL's procedure are: (4) Cultivation of cultures of seedlings grown from the seeds of individual wild plants. (5) Cytological examination and pollen analysis (recently adopted by HALL's co-workers). (6) Interspecific crossing, between closely related species, and between any two species whose natural ranges overlap and which might be expected to hybridize in nature.

All these procedures have been helpful, although the first was the least so because of the fragmentary nature of most rose specimens in herbaria. Herbarium specimens help to give an idea of the geographical distribution of the main rose types and also to give indications of possible localities for collecting trips in unfamiliar regions. Herbarium specimens of *Rosa* should be collected when the fruit is well developed and should consist of a segment of two-year-old wood, with two or three flowering branches bearing ripe or nearly ripe fruit, and a turion of the season. WOLLEY-DOD (**44**) has emphasized that "more than one specimen from each bush facilitates diagnosis." Good series of specimens from a single locality are also valuable, although not often to be found. The best series of local rose specimens in this country are those collected by SCHUETTE in eastern Wisconsin, now in the Field Museum; DEAM's specimens

from Indiana in the Deam Herbarium; and HOUSE's New York roses in the State Museum at Albany. There are no good representative collections of Pacific Coast roses, although the region from Alaska to central California is probably richer in species than is any other on this continent. The best generally representative collection is that at the University of Michigan.

Species in Rosa

Species of the Linneon type most certainly exist in the genus *Rosa*. Even a brief study of the genus brings out two important facts:

(1) A great diversity of extent among the species. Some species are delimited without difficulty, breeding true and showing only minor variability; others display an enormous degree of variation. In North America the former nearly all have a circumscribed area of distribution, while the latter extend for thousands of miles and may be called polymorphic species, collective species, or species complexes, because taxonomists have given specific rank to so many of their forms.

(2) The existence of a number of variable characteristics common to several of the large polymorphic species which show parallel series of varieties. This was recorded for *R. acicularis* Lindl. and *R. blanda* Ait. from field studies in northern Michigan (**12**).

One of the most satisfactory definitions of a Linnaean species is that given by VAVILOV, "a separate morpho-physiological system connected in its genesis with a definite environment and area" (**43**). This definition appeals to the experimentalist because it recognizes the physiological and geographical elements. It has been endorsed by BABCOCK, whose experimental work in taxonomy has confirmed the existence of "unit-groups" of organisms. These natural groups have been characterized in a broad way by BABCOCK (**3**) as possessing "relative stability, combined with a definite tendency to vary." This broad concept of the species is also subscribed to by CLAUSEN (**7a**).

The lack of good distinguishing morphological characteristics among the vast number of rose species, and the ease with which interspecific hybrids are obtained (even when the parents belong to different sections of the genus or differ greatly in chromosome number), are

indications that the genus has been in a rapid evolutionary phase, probably since the Pleistocene, and that most of the species are genetically not distantly related. Similar mutations appearing in different species of the same genus indicate a high degree of genetical similarity (**24**). The parallel series of variations of the polymorphic species provide further evidence for the assumption that the species of the Cinnamomeae and Caninae at least are closely allied to each other. This agrees with VAVILOV'S law of homologous series in variation, for he states (**42**) that "genetically nearly related Linneons have consequently similar series of hereditary variation."

If these hypotheses be accepted, they will account for the numerous species, varieties, and forms in *Rosa* and for the lack of agreement among taxonomists as to whether the species exist by the hundreds or by the thousands.

The taxonomist must find some way of describing all the multitude of natural forms, and in *Rosa* the problem of how to treat the units smaller than a species is perplexing. GREGOR (**23**) pointed out that if a varietal name is appended to every specimen which differs in some minor point from the general description of the group, then "every genotype and every clonal modification of an individual would ultimately deserve varietal or sometimes specific rank." To name all the possible combinations of minor characteristics leads to such a state of taxonomic inflation that the categories below the genus lose all practical value. Nor can the morphological criteria alone be accepted as supplying a completely reliable system of classification (**23**), for morphologically similar forms may differ markedly physiologically, as has been found in *R. acicularis*, *R. woodsii* Lindl., and *R. arkansana* Porter. Probably the most promising method for dealing with the smaller units is that of distinguishing minor variations by numbers or letters, which makes it possible to express different character combinations clearly and succinctly without degrading scientific nomenclature. This has been advocated by VAVILOV (**42**), HALL and CLEMENTS (**26**), CLAUSEN (**7**), and HALL (**25**). It was recommended for the varieties of rose species by MATTHEWS (**31**) and is a method that I also would endorse.

North American rose species in 1925

There has been a great multiplicity of species described for *Rosa*, especially in Europe, yet it is impossible to identify specimens by the keys of ROUX or WOLLEY-DOD (BOULENGER **5**). It is possible to describe an endless number of micromorphs in *Rosa*, but when this is done the taxonomic splitters continue to find forms in the field which do not fit any of their described species.

In America relatively few taxonomists have worked on the genus except to describe and to name specimens which were sent to the eastern centers by travelers from the west. The work of GRAY and WATSON belongs in this category. GREENE described several western species. He was a keen observer in the field and would often pick out isolated bushes which were striking in some particular and give them specific rank. RYDBERG believed that any individual that appeared different in detail should be described as a new species; yet he found that several of GREENE'S species were not distinctive enough in herbarium samples.

RYDBERG described a number of new species, almost entirely from herbarium material. He has given careful and painstaking analyses of the American species (**34**), and has discussed the relationships of some of them elsewhere (**33, 35, 36, 37**). Because he relied on herbarium material which was of very limited extent, he was often much concerned over characteristics that BOULENGER and I have found to be individual variations.

The work of CRÉPIN as it dealt with the American species was good, for he purposely tried to avoid multiplying species. Unlike RYDBERG, he could give varietal rank to plants varying in minor characteristics. He recognized parallel variation in rose species but frequently confused important and minor differences when diagnosing and differentiating species. He badly misunderstood some American species (see also RYDBERG **33**) and was handicapped by the fact that he worked too early to profit by the cytological data which has only more recently become available.

When this study was begun, there was such confusion between merely fluctuating and minor variations and differences of specific diagnostic value that almost any series of roses in the field or her-

barium presented some character combinations which had to be either described as new or left undetermined (**13**). The chief reason for this was the rigid specific descriptions, according to which a few more or a few less hairs or glands or prickles changed the scientific name of a specimen.

Work of Boulenger and Hurst

HURST'S unique theories, cultural experiments, and studies of *Rosa* have given valuable data on several characteristics. He has pointed out new ones, hitherto overlooked, such as the arching habit and the time taken to ripen fruit after flowering (**28, 29**). He also drastically limits the number of Linnaean species in the genus, which is a sound policy even though several of his groups are artificial.

BOULENGER'S work is based mainly upon a painstaking analysis of CRÉPIN'S large collection of roses in Brussels, together with field studies upon important characteristics. HURST, BOULENGER, and I all agree with CRÉPIN that there are stable recognizable species in *Rosa* and that the number of species conveniently recognizable in the genus is relatively small. I also subscribe to BOULENGER'S statement that, "dans ce genre si polymorphe, la distinction des espèces ne repose que sur des combinaisons de caractères qu'il est impossible d'exprimer en de courtes diagnoses" (**5**). What CRÉPIN (**8**) called "les caractères du portrait scientifique des espèces" must be given in lieu of a description of an ideal type. The analytical key which is appended to this paper often transgresses this rule. Yet it may be a better aid in this form to the inexperienced rhodologist, who should aim at writing a better one for his own local forms.

BOULENGER is an eminent authority in herpetology and ichthyology, and in taking up *Rosa* he hoped to be able to strike a new method of attack. He is disappointed in not having been able to do this; the chief thing he has had to do is to demolish, owing to the unreliable and injudicious splitting which has gone on.

CRÉPIN started work on the roses in 1860 and continued until 1900. In 1895 he announced that his monograph would be out in a year or two. Yet his notes were only on the herbarium sheets and in a chaotic state, two or three species names sometimes being placed on one sheet in the European forms. His determinations were often

full of provisions and he was never willing to commit himself. In 1925 BOULENGER suggested to me that it would be a useful contribution to American botany if someone could evaluate the characters used to distinguish species and even sections in RYDBERG's key (**34**), and to show that many of them are valueless as criteria. Such criticism, even though destructive, is necessary in order to keep old mistakes from being copied again.

BOULENGER strongly advocated the retention of large groups that have some amount of fixity, and when unusual forms are found, to describe them but without giving a name. When minor forms have been described as species it is sometimes expedient to keep the more outstanding ones as varieties, as he has done for *R. glauca* and *R. bakeri*, and as I have done for *R. brachycarpa* Rydb., even though this division has to be somewhat artificial.

Cytology and taxonomy of Rosa

The taxonomic difficulties of the genus *Rosa* are proverbial, chiefly owing to the unstable, fluctuating, or generalized nature of the characteristics that can be used for the diagnosis of species. In 1922 TÄCKHOLM'S (**39**) excellent treatise on the cytology of *Rosa* appeared, and it was hoped that the variation in chromosome number would solve the rhodologist's difficulties. Soon afterward HURST (**28**) proposed a purely artificial classification of the genus, based on chromosome number and upon the assumption of five primitive diploid species. His cytological assumptions have since proved to be invalid (**19, 21**). In my experience chromosome number has proved to be no more than another important diagnostic characteristic (**14**). Cytological studies have helped to clarify the situation, but have not seriously affected the classification of the genus as worked out by CRÉPIN in the last century and by BOULENGER on purely morphological lines more recently (**5**).

The American species fall into three main groups cytologically: the diploids with $2n = 14$, the tetraploids with $2n = 28$, and the hexaploids with $2n = 42$. The hexaploid species *R. acicularis* also has an octoploid race ($2n = 56$) which differs physiologically but scarcely at all morphologically from the more widespread (in America) hexaploid forms.

Pollen grain size varies directly with chromosome number (18) and provides a useful additional method for diagnosing non-typical variants, even in herbarium material.

Position of North American roses within the genus

The native North American rose species[2] fall into three distinctive and natural sections: (1) Synstylae (1 species), (2) Minutifoliae (3 species), and (3) Cinnamomeae (all other species). This agrees with CRÉPIN'S (10) arrangement except that his section Carolinae has been found not to be separated by any reliable characteristics from the section Cinnamomeae. *R. gymnocarpa* Nutt. is also now included in the Cinnamomeae.

The species of the section Cinnamomeae offer the greatest difficulties, being widespread and highly polymorphic. The four species in other sections,—*R. setigera* Michx. (Synstylae), *R. minutifolia* Engelm., *R. mirifica* Greene, and *R. stellata* Wooton (Minutifoliae),—are readily distinguishable. This paper deals with the section Cinnamomeae, unless otherwise stated.

Diagnostic characteristics in Rosa

It is not impossible to find on the same bush two of some of the species listed by RYDBERG (34). Moreover, four or five of these species may occur among the offspring of a single wild rose, as shown for *R. blanda* (14) and for other species in the appended tables of sib cultures grown at Pasadena. This is not a rare phenomenon but rather usual in the groups of *R. acicularis*, *R. arkansana*, *R. blanda*, *R. californica* S. & C. (fig. 1), *R. carolina* L., *R. durandii* Crépin, *R. pisocarpa* A. Gray, and *R. woodsii*, and would probably be discovered in other species if this method were followed.

These variable minor characteristics which give similar series of parallel variations in many American, Asiatic, and European rose

[2] Introduced species. The following exotic species are well established in North America (34):

*R. multiflora Thunb.	*R. laevigata Michx.	*R. micrantha Borrer
R. moschata Mill.	*R. bracteata Wendl.	*R. canina L.
R. sempervirens L.	*R. gallica L.	*R. cinnamomea L.
R. indica L.	*R. tomentosa Sm.	*R. pimpinellifolia L.
	*R. rubiginosa L.	

* Frequent in some districts.

species, therefore, should obviously be recognized as part of the variability of each species, and as useless in distinguishing between species or in determining hybridity.

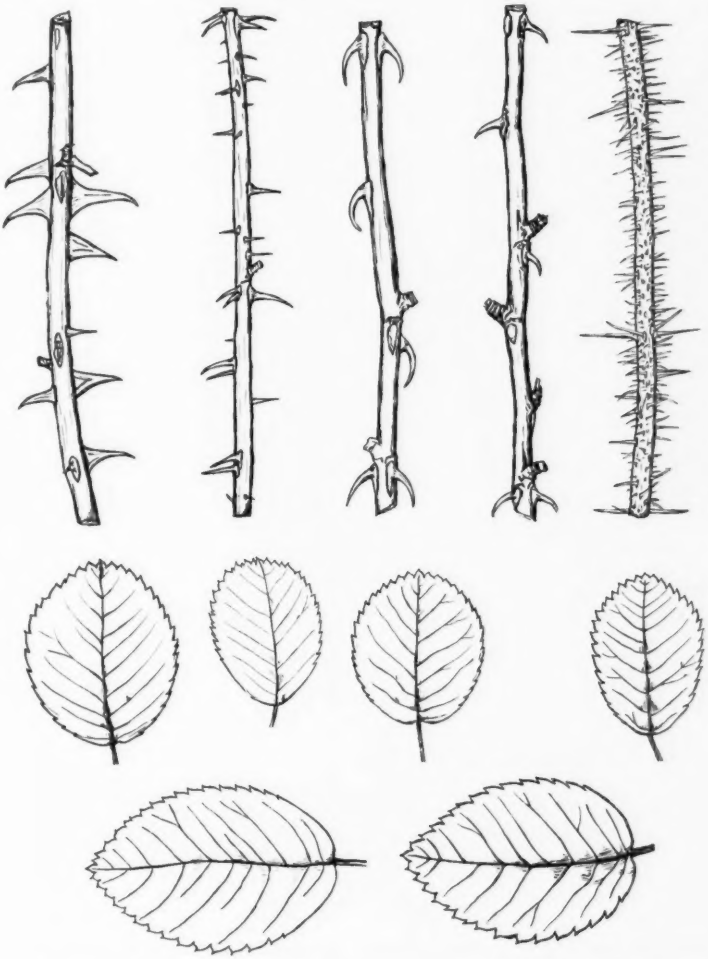


FIG. 1.—Types of stem armature and leaflets from single culture of *R. californica* (no. 12307) grown at Pasadena, Calif. (nat. size.)

RELIABLE DIAGNOSTIC CHARACTERISTICS

Some good diagnostic characteristics are physiological and therefore are not admissible by the conventional taxonomist. Lack of good characteristics demands their use as an aid in delimiting species:

1. TIME OF FLOWERING.—Among a collection of species under uniform conditions, or in one locality, this is an important and useful diagnostic character (16). Both BOULENGER (5) and I have inde-

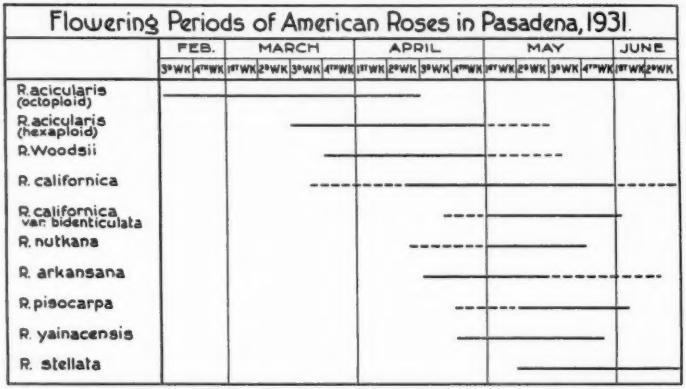


FIG. 2.—Flowering periods of each of 10 native species of *Rosa* at Pasadena in 1931. Main flowering period plotted as unbroken line; stippled lines indicate a few scattered flowers before and after main flowering period.

pendently found that the morphologically simple species flower first and have a more boreal range than have species with more advanced characteristics. The octoploid *R. acicularis* can be distinguished from the hexaploid variety on this account (fig. 2). *R. bidenticulata* Rydb. has also been retained for the reason that it flowers later than *R. californica*.

2. HABITAT.—*R. acicularis*, *R. blanda*, *R. pisocarpa*, and *R. woodsii* belong to littoral habitats (14). *R. arkansana*, *R. californica*, *R. carolina*, *R. durandii*, *R. foliolosa* Nutt., *R. gymnocarpa*, *R. nutkana* Presl., and *R. spithamaea* S. Wats. are more usually found in upland or prairie habitats. *R. nitida* Willd., *R. housei* Erlanson, and *R. palustris* Marsh. are found in bogs. This habitat preference distin-

guishes *R. housei* from its western relative *R. arkansana*. When grown at Ann Arbor, one plant of *R. housei* (13207)[3] assumed the normal habit of *R. arkansana* and produced flowering turions with terminal corymbs. It is perhaps an ecophene (**40**), but more material must be grown to confirm this.

3. HARDINESS AND CLIMATIC TOLERANCE.—Cultures at the Botanical Gardens of the University of Michigan showed that some of the roses of the Pacific Coast region were only partially hardy in southern Michigan. *R. californica*, *R. pisocarpa*, and *R. gymnocarpa* grew very slowly, seldom flowered, and were often cut back by frost or winter killed. *R. woodsii* was very variable in this respect. Plants or seedlings from British Columbia, Washington, the Rocky Mountains, and the Great Plains thrive in Michigan. Plants or seedlings from the arid Great Basin persisted in Michigan but were stunted; they lost their leaves during the summer drought and never flowered. These two physiologically different groups within *R. woodsii* show a parallel series of variations, and cannot be distinguished morphologically.[4]

R. acicularis from Alaska ($2n = 56$) responds so rapidly to a rise of temperature in spring that the flower buds are almost always completely frost-killed in April in southern Michigan. The hexaploid requires more or prolonged warmth and always flowers profusely. *R. nutkana* thrives in Michigan, but was stunted and put out little growth in southern California. It also came into leaf later there, in comparison with *R. acicularis* and *R. woodsii*, and flowered later than *R. woodsii*, synchronously with *R. californica* (fig. 2), although in Michigan it flowers earlier than these last two species (**16**). *R. pisocarpa* was also considerably behind *R. woodsii* in coming into leaf at Pasadena and flowered later than that species.

Seedlings of *R. blanda* from northern Michigan segregated into tender and hardy individuals at Ann Arbor, 300 miles south of their natural habitat. It is interesting to note that the hardy strains of

[3] Culture numbers represent accession numbers at the Botanical Gardens of the University of Michigan.

[4] *R. rubiginosa* and *R. setigera* grow to their normal height, flower profusely, and set much fruit at Ann Arbor, although they are more or less seriously cut back by winter temperatures in some seasons.

R. woodsii seem not to have persisted in the region of the Great Basin. Plants from the Mojave desert and from southern New Mexico have not been tested at Ann Arbor. Abortion of hypanthia was frequently observed in the arid regions of the west (**15**) and may well have been due to lack of water. JOHANSEN (**30**) found that in *Zauschneria* in California "insufficiency of water underlies the degeneration of the megagametophytes."

The preceding instances all illustrate a feature of what GOOD (**22**) has called the theory of tolerance; that is, that "morphologically similar species may show wide differences in tolerance." Such characteristics as these, of course, are of little or no aid in the herbarium.

Concerning the morphological characteristics that are valuable I agree with BOULENGER and with HURST:

1. HABIT.—The primitive, strict, straight type of branching is common to most of the American species (fig. 16).

R. palustris has zigzag lateral branches.

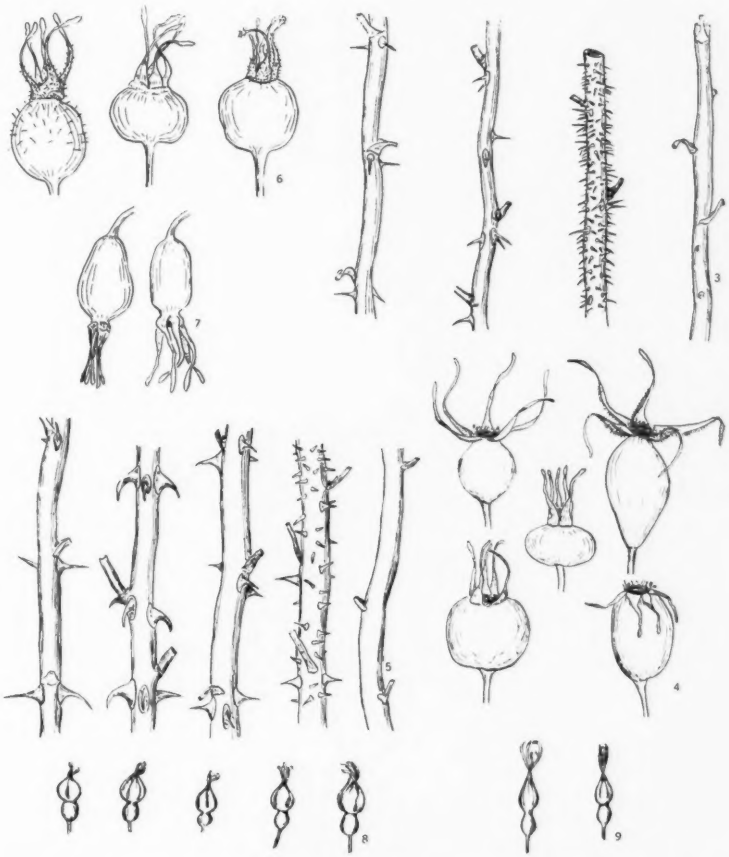
R. arkansana and *R. foliolosa* are usually subherbaceous, or suffruticose, as are some individuals of *R. carolina* and *R. rudiuscula* Greene, with weak decumbent stems.

R. californica and *R. pisocarpa* usually have the subcernuous branches which are stressed by HURST in his EE septet series (figs. 14, 15) although some individuals with the strict habit segregated out in several of my cultures (fig. 17).

2. FOLIAGE.—The *number of leaflets* can be used only in some cases. Leaflets 5–7 on two-year wood and 7–9 or 11 on first-year growth are usual (table I). A few species have been found to have not more than nine leaflets. Ovate leaflets with slightly cordate base are characteristic of *R. pisocarpa* and sometimes of *R. californica* (figs. 1, 10, 11). Lanceolate or elliptic leaflets are usual in *R. foliolosa*, *R. nitida* Willd., and *R. palustris*, but leaflet shape is an extremely variable and unreliable character. All seedlings have glabrous foliage with 3–5 leaflets for the first four or five nodes, and the leaflets are glabrous with glandular-serrulate teeth.

The *number of teeth* per leaflet increases with more highly evolved forms, in BOULENGER'S experience. *R. palustris* alone shows a consistently high number of usually fine, simple teeth, among the roses of the Cinnamomeae (**12**). *R. pisocarpa* has fine crenate teeth; those

of *R. woodsii* are ascending serrations, usually coarse and acute (fig. 12).



FIGS. 3–9.—Fig. 3, stem armature in *R. blanda;* fig. 4, hip shapes in *R. blanda;* fig. 5, types of stem armature in *R. palustris;* figs. 6, 7, hip shapes in *R. acicularis;* fig. 8, five flower buds of *R. californica;* fig. 9, flower buds of *R. woodsii.* (nat. size.)

Stipules are for the most part unimportant for diagnostic purposes. They are characteristically narrow in *R. foliolosa*, *R. palustris*, and *R. californica*, and dilated in *R. blanda* and *R. woodsii*.

3. LENGTH OF FLOWERING LATERALS.—This is correlated with time of flowering and with size of inflorescence. The early flowering *R. acicularis*, *R. nutkana*, and × *R. engelmannii* S. Wats. have flowering laterals that are usually 3–10 cm. long. All the other species have some laterals about 7 cm. long and some that may be twice that length. Roses that flower terminally on suckers (flowering turions) may also produce laterals from the base of the main stem which grow to the height of the main plant before flowering.

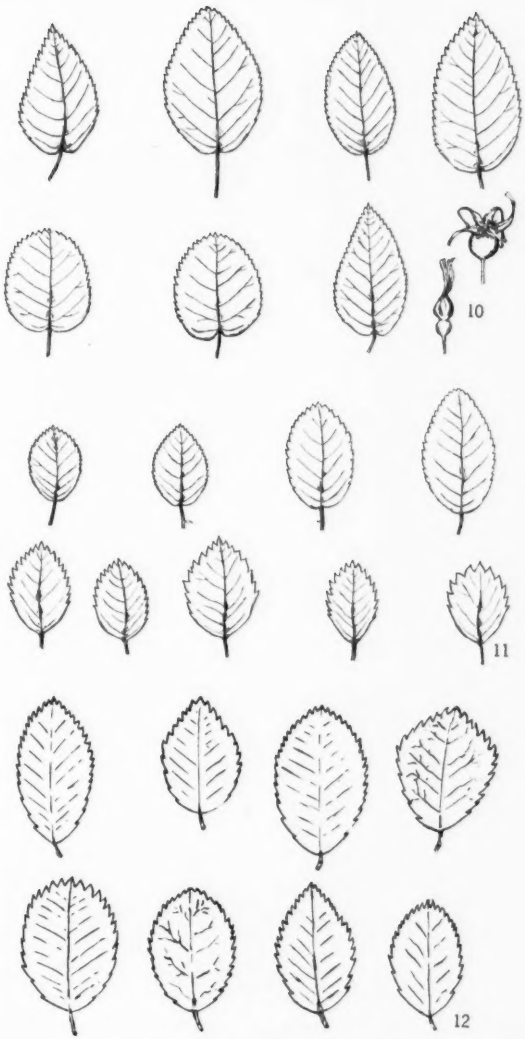
The production of flowering turions after the main flowering period gives what horticulturists call the everblooming habit, and is a valuable diagnostic characteristic for *R. rugosa* Thunb. and for most of the tetraploid American species (except *R. durandii* and *R. yainacensis* Greene).

4. INFLORESCENCE.—Early flowering roses have solitary flowers or 2–3 together on short laterals (for example, *R. acicularis* and *R. nutkana*). The other species of the Cinnamomeae sometimes produce some flowers 1–3 together on short laterals, and others in small or large cymes (4–20 flowers) on longer laterals (table I). A group of flowers subtended by a simple bract, or by a bract with a single terminal leaflet, is considered as a single cyme. Three or four small cymes may cluster at the end of a single lateral, each subtended by a true foliage leaf.

5. LENGTH OF PEDICEL.—This is variable and not of much value, although some species always have stout erect pedicels in fruit. In others the pedicels are erect or pendent in the same culture, even on the same plant.

6. SEPALS.—Width of sepals can be used to differentiate between *R. woodsii* and other diploid species. *R. woodsii* has linear-lanceolate sepals 1.5–2.5 mm. wide at the base (fig. 9). No other sepal character has been found to be reliable. In the species of the Cinnamomeae the sepals have almost straight sides; only in *R. californica* (figs. 8, 10) and *R. pisocarpa* is there a tendency for them to be somewhat constricted at the base.

7. PETALS.—The northern and eastern roses and those of the Great Plains have petals 2 cm. or more in length. The Oregon and California species (except *R. nutkana*) have petals usually under 2 cm. long (table I).



FIGS. 10–12.—Fig. 10, leaflets, flower bud, and ripe hip from culture of *R. pisocarpa;* fig. 11, leaflet shapes in one culture of *R. californica* (top row) and *R. californica* var. *bidenticulata* (bottom row); fig. 12, leaflets of *R. woodsii.* (nat. size.)

TABLE I

NUMERICAL VARIATIONS FROM CULTURE EXPERIMENTS

COLUMNS: 1, NUMBER OF LEAFLETS ON WELL DEVELOPED LEAVES; 2, LENGTH OF TERMINAL LEAFLETS; 3, NUMBER OF TEETH ON EACH SIDE OF TERMINAL LEAFLETS (AVERAGE NUMBER IN PARENTHESES); 4, LENGTH OF FLOWERING LATERALS; 5, NUMBER OF FLOWERS IN AN INFLORESCENCE; 6, LENGTH OF PEDICELS; 7, SEPAL WIDTH; 8, PETAL LENGTH; 9, NUMBER OF STAMENS (AVERAGE NUMBER IN PARENTHESES); 10, DIAMETER OF HIP

1	2 (CM.)	3	4 (CM.)	5	6 (CM.)	7 (MM.)	8 (CM.)	9	10 (MM.)
R. blanda									
5–9	1–5.7	5–20 (12)	3–25	1–20	1–3	2.5–4	2–2.8	85–140 (115)	8–15
R. woodsii									
5–11	0.5–4	5–24 (12)	2–20	1–18	0.3–2	1.5–2.5	1–2.5	35–85 (65)	8–12
R. pisocarpa									
5–9	1–4	8–25 (15)	3–10*	1–9*	0.5–1.5*	2–4	1.2–1.7	44–103 (75)	7–10
R. californica									
3–9	1–4	10–20 (14)	3–30	1–26	0.3–2	3–4.5	1.5–2	65–123 (90)	8–15
R. californica var. bidenticulata									
3–7	0.5–2.5	4–14 (8)	1–10	1–6	0.3–1	3–4	1.2–1.8	65–98 (77)	8–10

* Probably smaller than normal because of dry atmosphere of southern California.

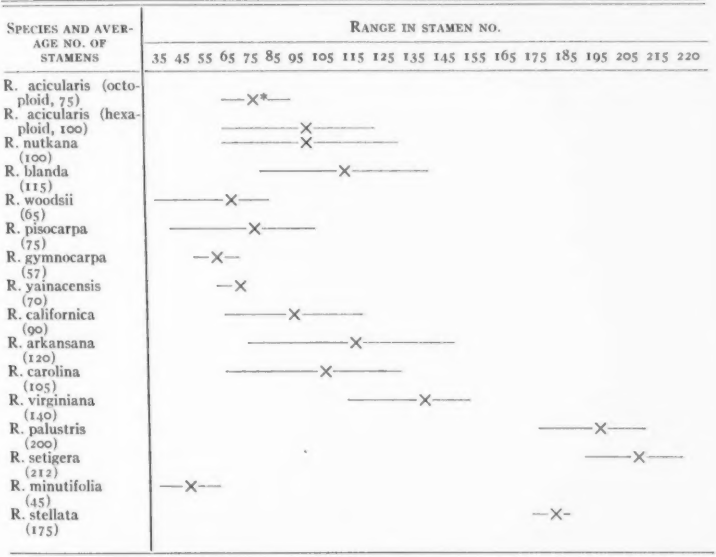
8. STAMENS.—The number of stamens in each species varies, in some instances within a wide range. Stamen number, however, is a reliable specific character. In *R. virginiana* Mill. the number of stamens is fairly constant and markedly higher than in *R. carolina*.

R. palustris has a higher number of stamens than has any other rose of this section. *R. setigera* has an average of 200 stamens (table II).

9. ACHENES.—The number of achenes is roughly 25–50 per cent of the number of stamens. *R. gymnocarpa* has few large (5×3 mm.) usually glabrous achenes. *R. palustris* has numerous small (1.5 mm. long) achenes, which float in water (**17**).

TABLE II

NUMBER OF STAMENS IN AMERICAN ROSES


SPECIES AND AVERAGE NO. OF STAMENS
RANGE IN STAMEN NO.
35 45 55 65 75 85 95 105 115 125 135 145 155 165 175 185 195 205 215 220
R. acicularis (octoploid, 75)
R. acicularis (hexaploid, 100)
R. nutkana (100)
R. blanda (115)
R. woodsii (65)
R. pisocarpa (75)
R. gymnocarpa (57)
R. yainacensis (70)
R. californica (90)
R. arkansana (120)
R. carolina (105)
R. virginiana (140)
R. palustris (200)
R. setigera (212)
R. minutifolia (45)
R. stellata (175)

* × indicates the average number.

10. DISC AND URCEOLE.—These do not show any significant interspecific differences among the American Cinnamomeae, although BOULENGER (**5**) found them valuable in distinguishing between some European species.

MINOR AND UNRELIABLE PARALLEL VARIATIONS

1. HEIGHT OF STEM.—This is a characteristic that must be treated carefully. It has been found that many of our species segregate dwarfs in culture (fig. 18), so that dwarfness is not a diagnostic char-

acteristic. Some species seldom reach over 1 m. in height, however, whereas under favorable conditions others may attain 2–3 m. or more.

2. ARMATURE.—There are three main types of armature:

(a) Stems normally bristly. A seedling and primitive armature type (**5**). For example, *R. acicularis*, *R. arkansana*, *R. blanda* (fig. 3).

(b) Stems with bristles and usually terete slender prickles intermixed: heteracanthic. For example, *R. californica*, *R. gymnocarpa*, *R. rugosa*, *R. woodsii*.

(c) Stems with well developed prickles that are usually strong, flattened, and enlarged at the base. These plants fall into two groups: (1) Bristles and stout prickles intermixed throughout: heteracanthic; for example, *R. californica*, *R. durandii*. (2) Bristles usually absent except in the first season and at the base: homoeocanthic. The only true homoeocanthic North American species is *R. setigera;* in the Cinnamomeae, *R. nutkana*, *R. palustris*, *R. pisocarpa*, and *R. virginiana* approach this condition (**5**, II, figs. 7 and 8 for *R. canina*).

A plant of any armature type may sometimes be completely unarmed; again, only the twigs may be unarmed, or the armature may appear on the lower half of the plant only.

3. FORM AND DIRECTION OF PRICKLES.—The bristles and prickles of any of the armature types may be straight, ascending, reflexed, or hooked (fig. 5, *R. palustris*). Prickles on the same bush frequently vary in form and direction, so that it is useless to distinguish species by this characteristic. Armature is largely a matter of degree.

4. INDUMENT.—The presence or absence of hairs, glandular hairs, or glandular granules on stem, foliage, stipules, hypanthia, pedicels, or sepals is found in varying degrees in all the species. This was reported by SCHNETZ (**38**) to be an individual peculiarity in roses, but is still used in keys. Simple or compound serrations on the margins of leaflets and gland-tipped serrations often vary on different parts of the same bush, and on different sibs of cultures. Some species are reported not to have glands on the leaflets (except as seedlings); for example, *R. cinnamomea* L., *R. rugosa*. Should a plant of either be found with glandular foliage, however, this would not exclude it from

the species, because this type of covering is a regular part of the parallel variation of the genus. *R. palustris* and *R. virginiana* have not been found with glands on the leaflet surfaces, although an example of each has been observed in herbarium material with finely gland-serrulate leaflets.

5. SHAPE OF RIPE HIPS.—A cursory examination of any series of living roses will show that the hips vary from depressed globose to globose, elliptic, pyriform, and urceolate, and that they often vary on individual bushes and among plants belonging to the same species. In no American species is there a single hip shape. This characteristic is of almost no practical value in distinguishing species (figs. 4, 6, 7). BOULENGER told me that there is not a single rose group in Europe in which a species can be based upon the shape of the fruit (see also BOULENGER **5,** I).

6. POSITION OF SEPALS ON RIPE HIP.—As shown in the tables, sepals may be erect, spreading, or reflexed on the full-grown hips in different individuals of the same culture in *R. woodsii*, *R. pisocarpa*, and *R. californica*. This is also true for different hips on the same plant in several groups. The Carolinae were distinguished from the Cinnamomeae largely because their sepals were supposed to be reflexed and deciduous in fruit. In *R. palustris* (section Carolinae of authors) sepals frequently remain erect and do not always fall. Plants of *R. woodsii* and of *R. pisocarpa* (fig. 10) (section Cinnamomeae) have been observed with spreading and deciduous sepals.

7. POSITION OF ACHENES ON WALL OF HYPANTHIUM.—This character was used by CRÉPIN to distinguish further between the sections Carolinae and Cinnamomeae. In *R. palustris* achenes are frequently attached to the side wall of the hypanthium. Plants can be found in *R. blanda*, *R. woodsii*, *R. pisocarpa*, and other species of the Cinnamomeae with achenes at the base of the hypanthium only. BOULENGER (**6**) also found this to be an inconstant character.

Experimental tests of status of some American species

It is difficult to present convincing evidence for the existence of variations on single individuals in prickle direction and form, leaf covering, glandulosity, sepal position, and hip shape, although anyone can quickly convince himself by inspecting living rose bushes.

In this paper an attempt will be made to prove the unsound nature of such characters for distinguishing between species, by tabulating the combinations of those that appeared in cultures of seedlings from single wild female parents. The objection might be offered that these various strains were kept alive by cultivation but would die out in nature. This can hardly be sustained when we remember that these are all minor variations with no apparent survival value (unless dwarfness might be excepted); and that nearly all the variants were observed in nature, sometimes in small areas (**12, 15**).

A second striking fact, beyond the existence of parallel series of variations in several of the collective species, is the existence of a variable amount of gametic sterility and of chromosome aberrations among some members of almost any species. All these phenomena have been attributed to hybridization between different species in nature, an assumption which needs to be reconsidered in the light of recent data.

VARIATION IN PROGENY OF WILD ROSES

R. blanda Ait.—The segregations found among the progeny of a wild plant of *R. blanda* var. *glandulosa* Schuette have been reported (**14**). Since then a more complete analysis of the largest culture (3753/7) has been made (appendix I, table A). It contains plants which would be classified in the field or herbarium as *R. blanda*, *R. subblanda* Rydb., *R. blanda* var. *glandulosa*, *R. blanda* var. *hispida* Farwell, *R. acicularioides* Rydb., and *R. palustriformis* Rydb. These four species and two varieties are distinguished solely by one or more of the minor variable characteristics which have been listed under "unreliable parallel variations."

In order to give, at a glance, the variation discovered in such a culture, these characteristics have been designated by a system of numbers. The numbers have been grouped in columns headed by letters which stand for the part of the plant dealt with (table A). In this way the formula for any individual plant can be written in brief form and can be compared with that of any other. The explanation of the symbols and the formulas for some individual cultures are given in appendix I. Each individual has been determined in most cases by RYDBERG's key (**34**), and the binomial name thus obtained is given in the last column.

The result of pollen and achene analysis in culture 3753/7 of *R. blanda* showed a great variation in sterility. Figure 13 shows the

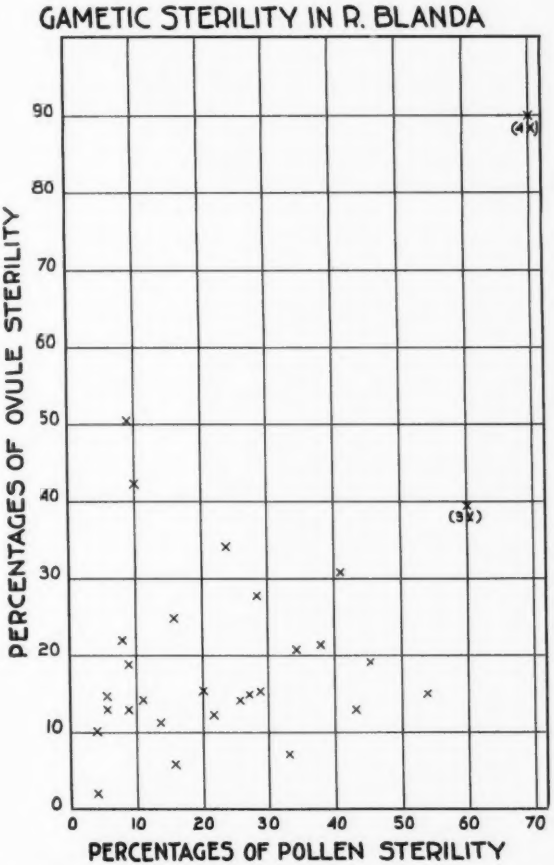


FIG. 13.—Ovule and pollen sterility in 31 plants of *R. blanda*, from culture 3753/7 plotted synchronously for each plant. Data taken in 1930.

male and female sterility percentages for 31 plants, plotted synchronously. No correlation is brought out. Apomixis was found once to occur in the plant shown on the chart with 53% sterile pollen and 15% empty ovules, but has not been obtained again in this plant nor

in any of its sibs. Among 51 plants, 40 had 4–33% of the pollen grains empty, 10 had 34–65% empty pollen, and one plant had 66% bad pollen. Five plants had 50% or more of bad pollen, and of these three were diploid ($2n = 14$), one was triploid ($2n = 21$), and one was tetraploid ($2n = 28$). The semisterile diploid plants showed multivalent groups of chromosomes at diakinesis (**19, 21**), owing to reciprocal translocations. This is now known to be a widespread phenomenon and is usually not a result of interspecific hybridization. The triploid plant is trivalent in synapsis and apparently arose from the union of a haploid and an unreduced diploid gamete (**21**). The tetraploid plant flowers first in the culture (it is a dwarf) and seldom sets fruit. This plant probably originated in the union of two unreduced gametes. The female parent of this culture is diploid and has only 15% of bad pollen. This is another example of the rule announced by DARLINGTON (**11**) that the fertility of a tetraploid is inversely proportional to that of the diploid from which it arises.

The type specimen of *R. blanda* is a small unarmed spray, yet in all parts of its range this rose is frequently bristly and sometimes has weak infrastipular prickles (fig. 3). Diploid *R. blanda* sets a good crop of achenes with pollen from hexaploid *R. acicularis*, and because these two species occur together in the Great Lakes region I at first attributed the great variability of *R. blanda* in northern Michigan to hybridization with *R. acicularis*. Since then a culture of seedlings has been raised from a plant of *R. blanda* from the Gaspé Peninsula, Quebec, where *R. acicularis* is not found. They also show great variability (appendix I, table B); they are nearly all bristly but do not have the elliptical pendent type of hip common in northern Michigan. Several plants in the Canadian culture have bristles on the hypanthium, a characteristic that is one of individual variation in *R. blanda* as in *R. acicularis* (fig. 6), *R. carolina*, *R. nutkana* (**13, 15**), and other roses (**5**).

R. woodsii Lindley.—In the Great Basin of Utah and Nevada, the common roses at low altitudes all belong to the large diploid group of *R. woodsii*. Species belonging to other groups are not found in Nevada; and in Utah, although hexaploid forms occur, they are limited to high altitudes. In 1928 seeds were collected in these regions (**15**), and the offspring of several individuals were grown at the experi-

mental garden of the California Institute of Technology in Pasadena, at the invitation of DR. T. H. MORGAN. Most of these seedlings flowered for the first time in 1931 and were then examined. If the variability of *R. blanda* were due to hybridization with other species, then one would expect greater uniformity among the offspring of the western diploids, which are isolated geographically.

The offspring of plants which were identified in the field by RYDBERG's keys as *R. fendleri* Crépin, *R. macounii* Greene, *R. puberulenta* Rydb., *R. pyrifera* Rydb., *R. salicetorum* Rydb., and *R. woodsii*, not only showed as much variation as those of *R. blanda* but had the same variations morphologically. Single cultures from Utah and from Nevada contained some plants with glabrous, some with pubescent, and others with glandular foliage. Among offspring of single plants some were almost unarmed, others were densely bristly with weak paired prickles. Straight and curved prickles, glabrous and glandular sepals, and globose and pyriform hips also appeared in the same cultures (appendix I, tables C-J). In the fragments found in herbaria, each of these variants had been given a separate specific name; consequently, when other combinations were found they had to be described as new species.

Nine offspring of a single plant (culture 12191) from Salt Lake City can be placed in seven different species according to RYDBERG's keys. In four of the six plants that flowered in 1931, more than one-third of the pollen grains were empty. A culture of 21 plants from Nevada (12205) could be classified in five species; among 15 plants that flowered, six had less than one-third of bad pollen (table III), eight had approximately 50% bad, and one had 90% bad and was a triploid from the union of one unreduced diploid and one haploid gamete (**21**). Giant pollen grains (probably diploid grains) were observed in the pollen of three of the semisterile plants (table IV). Two of the semisterile plants which were examined cytologically showed quadrivalent groups in a few of the microspore mother cells at diakinesis (**21**). The eleven offspring of another plant (12207, which grew near 12205) could be classified in three species, and the pollen of the four examined showed only 5–12% of empty grains. Culture 12209, also from near Reno, contained 52 plants that could be classified in six species. Among ten plants that flowered in 1931,

seven had less than 33% bad pollen, two had about 50%, and one was a triploid with 88% bad pollen. Similar variations were shown in other cultures from this region (appendix I). A small percentage (0.5–0.6%) of giant grains was observed in pollen of plants with only 20–29% of empty grains.

TABLE III

VARIATION IN POLLEN STERILITY IN CULTURES OF R. WOODSII (RANGE 3–95%)

CULTURE	PERCENTAGE		
	0–33	34–65	66–100
12191	2	4	.....
12205	6	8	1*
12207	4†	.....	.....
12209	7	2	1*
12211	.....	2	.....
12212	1	.....	.....
12218	1	1	.....
12220	7	3	.....
12227	1	.....	.....
Totals	29	20	2

* Triploids. † Range, 5–12%.

TABLE IV

OCCURRENCE OF GIANT POLLEN GRAINS IN CULTURES OF R. WOODSII

CULTURE	NUMBER OF PLANTS	DEGREE OF STERILITY	PERCENTAGE OF GIANT GRAINS
12191	2	Semi	0.3–0.6
12205	2	Semi	1.4–2.1
12220	1	20%	0.59
12221	1	29%	0.6
12227	1	25%	0.6

R. pisocarpa A. Gray.—This species did not thrive at Pasadena, so that the length of flowering laterals and size of inflorescence were less than would be normal in a moister region. The cultures varied less than those of *R. woodsii* and *R. californica*, but sufficiently to show that absence of armature, hip shape, indument of sepals, and

position of sepals on the fruit are unreliable characteristics on which to base specific differentiation (appendix I, tables K-O). *R. anacantha* Greene, *R. copelandii* Rydb., *R. eastwoodiae* Rydb., *R. pringlei* Rydb., and *R. ultramontana* (S. Wats.) A. Heller are distinguished from *R. pisocarpa* by such characteristics. Among 23 pollen analyses that were made from all cultures, 14 plants had less than 33% bad pollen, six had between 50% and 60%, and three had approximately 70%. These last three were all triploids.

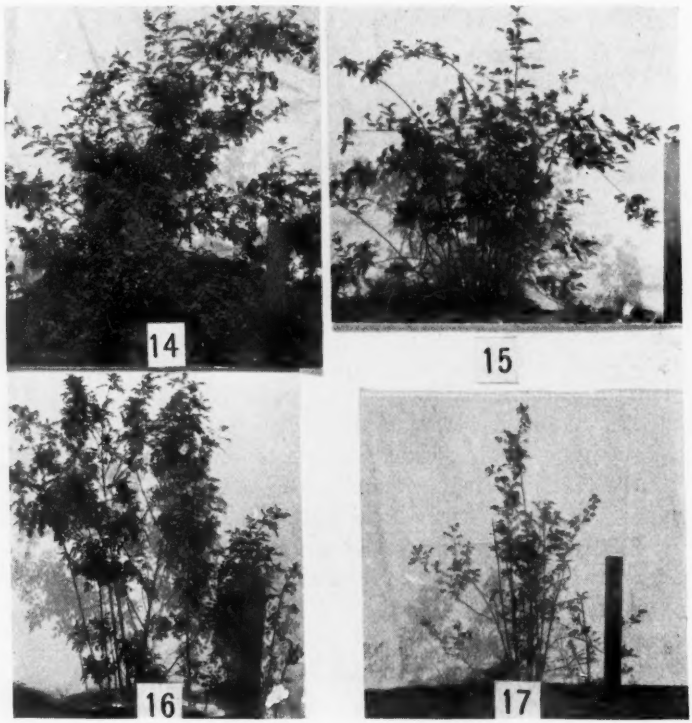
R. californica S. & C.—The tetraploid species *R. californica* showed great variation in minor characteristics and gave a series of variants parallel with those found in *R. woodsii*. The stem armature and covering of foliage are more complicated and more intergradations are possible in this group. Some dwarfs appeared in each culture, and a few segregates with strict instead of arched branches (figs. 14 and 17, and appendix I, tables P, Q, R, S).

Culture 12307 contained 44 plants, offspring from a single bush near Upland, San Bernardino County, California. The offspring had a fairly uniform appearance, except for two dwarfs. Yet when details of armature, leaflet, sepal and pedicel covering, and fruit shape were analyzed, as they would be in herbaria, the plants could be classified (with varying degrees of certainty) as belonging to ten of the species given by RYDBERG (**34**). It is no wonder that California botanists have not taken up these species, but prefer, correctly as I believe, to call them all *R. californica*.

Two small cultures from the San Francisco Bay region showed five plants out of six with about 50% of the pollen shriveled. Thirty plants out of thirty-one that were examined in the culture from Upland had less than 25% of empty grains; one plant had 35%. Variability in the minor characteristics is therefore not necessarily correlated with gametic sterility.

A small culture (12314) from a plant at Hesperia, San Bernardino County, California, consisted of plants about 7 dm. high with slender strict stems (fig. 17). The prickles on all were slender and terete, and leaflets were small, usually about 1.5 cm. long, glandular, and coarsely toothed with usually less than ten serrations each side (fig. 11) (appendix I, table S). These plants were tetraploids and resembled some of the dwarf segregates in cultures of *R. californica*. They were

strikingly distinct in habit from typical *R. californica* and agreed with RYDBERG's description of *R. bidenticulata*. RYDBERG's type was from Shasta County, and it is possible that this depauperate form is an ecotype of *R. californica* adapted to higher altitudes. Until more is



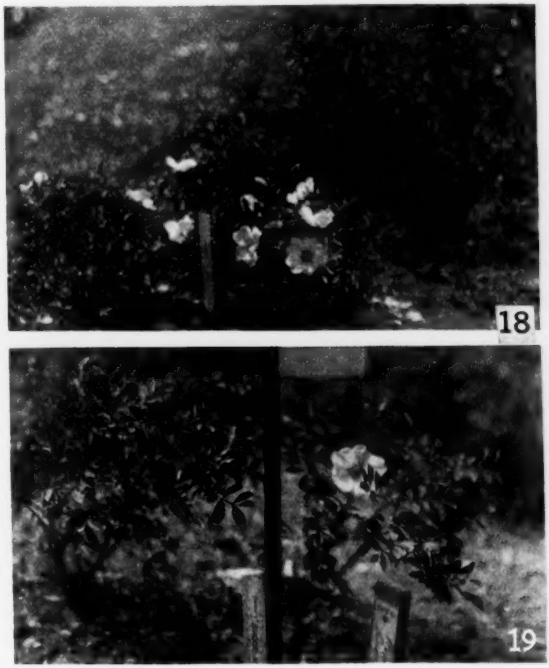
FIGS. 14–17.—Fig. 14, subcernuous habit of *R. californica;* fig. 15, subcernuous habit of *R. pisocarpa;* fig. 16, *R. woodsii*, strict habit; fig. 17, *R. californica* var. *bidenticulata* with strict habit (photographs by DR. G. W. BEADLE, at Pasadena, Calif.).

known about its range and variability, it is advisable to retain it as a variety, *R. californica* var. *bidenticulata* (Rydb.), comb. nov. (*R. bidenticulata* Rydberg, North Amer. Flora **22**: 518–519. 1918).

Other species.—The preceding examples demonstrate so clearly the fact of similar parallel variations of minor characteristics in different

rose species that details of the analysis of cultures will not be given for other groups.

There are cultures at the Botanical Gardens of the University of Michigan which show the same thing in *R. acicularis*, *R. arkansana*, *R. durandii*, *R. carolina*, *R. palustris*, and *R. virginiana*.



FIGS. 18, 19.—Fig. 18, tall and dwarf plants in single culture of *R. carolina* at Ann Arbor, Michigan; fig. 19, *R. alcea*, dwarf ecotype species related to *R. arkansana*.

In cultures of *R. acicularis* plants appeared that would be classified as *R. acicularis* var. *lacorum* Erlanson, *R. acicularis* var. *rotunda* Erlanson, *R. acicularis* var. *sayiana* Erlanson, *R. bourgeauiana* Crépin, and *R. collaris* Rydberg.

R. ratonensis Erlanson 1928 when self-pollinated gave plants answering to the description of *R. arkansana*, *R. conjuncta* Rydberg, *R. ratonensis*, *R. suffulta* Greene, and *R. suffulta* var. *valida* Erlanson.

Two out of four plants obtained from selfing the dwarf *R. relicta* Erlanson could be classified as *R. suffulta*, one as *R. arkansana* (which set no fruit after flowering), and one as *R. rudiuscula* Greene.

Seedlings in a culture of *R. durandii* from Oregon have not yet flowered, but they show great variation as to covering. Some have pubescent stems and prickles, others are glabrous. Some are very prickly and one almost unarmed.

Cultures of *R. carolina* from individual wild plants contained offspring that could be classified (according to RYDBERG) as *R. gemella* Willd., *R. lyoni* Pursh, *R. nanella* Rydb., *R. petiolata* Rydb., *R. serrulata* Raf., and *R. subserrulata* Rydb.

Cultures of *R. palustris* sometimes include specimens that agree with the descriptions of *R. dasystema* Raf., *R. floridana* Rydb., and *R. obtusiuscula* Rydb.

Cultures of *R. virginiana* show less variability. Some plants are low growing; others may have somewhat pubescent foliage (*R. lyoni* (**35**) or somewhat pyriform fruit (*R. bicknellii* Rydb.).

Field studies have shown that these variations and many others and numerous recombinations of characteristics also occur in nature in the preceding species as well as in *R. gymnocarpa* and *R. nutkana* (**12, 14, 15**).

BOULENGER, working with the extensive herbarium material of CRÉPIN, has found that *R. alpina* L., *R. pimpinellifolia* L., and others also show these same minor morphological variations. His observations have constrained him to reduce numerous so-called species to synonymy.

Culture experiments and field studies indicate that there are relatively few rose species in North America, but that these are highly variable and heterozygotic for the minor characteristics.

Hybridization

Hybridization has undoubtedly taken place freely between species of the section Cinnamomeae. The whole question of natural interspecific hybridization in *Rosa* is highly speculative (**5**, II). Intermediate types have usually been judged as hybrids, and the supposed identity of the two parents guessed at (**34, 35, 36**).

Several interspecific pollinations have been made at the University

of Michigan. Thanks to the cooperation of Dr. William Crocker and the staff of the Boyce Thompson Institute for Plant Research, who germinated most of the hybrid achenes, several good cultures of F_1 plants have reached maturity in the past two seasons. These crossings were mostly made in 1929 and 1930. Pollen has been ob-

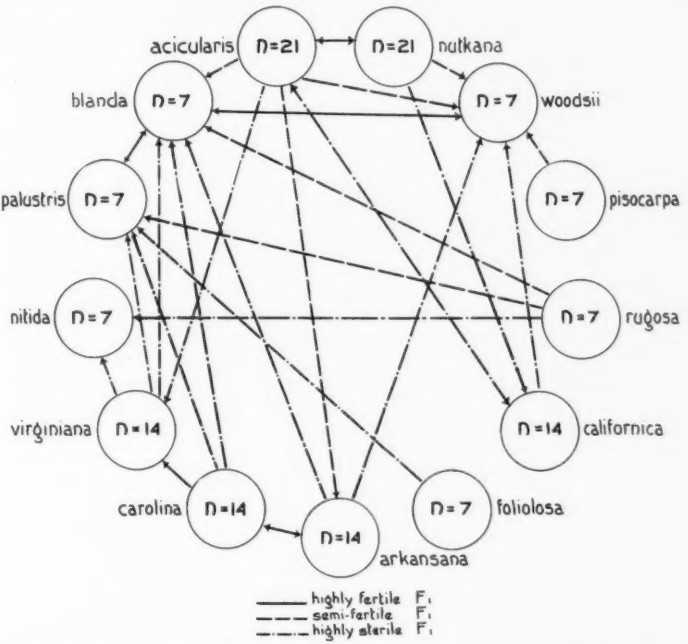


Fig. 20.—Diagram of hybridizations in section Cinnamomeae. An unbroken line shows that resulting F_1 has 1/3 to 2/3 of pollen empty; line of dots and dashes shows that the F_1 has almost all bad pollen; arrows indicate direction in which pollen was carried in making the cross.

tained from several of these interspecific hybrids, which may be conveniently arranged in three classes with regard to pollen fertility: (1) fertile, less than one-third bad pollen; (2) partially sterile, one-third to two-thirds bad pollen; and (3) sterile, over two-thirds bad pollen. These results concerning 13 species are shown diagrammatically in figure 20.

The hybrids will be analyzed in detail as they reach complete ma-

turity. Figure 20 shows that the fertility of hybrids between species having the same chromosome number cannot be predicted. Species possessing the same chromosome number that grow together in nature usually give highly fertile F_1 hybrids, and such hybrids are frequent enough in some regions to be recognizable in the field. BOULENGER (**5**, II) would eschew the practice of giving binomial names to hybrids, because the diverse forms shown by many of them tend to lead to an ever increasing multiplication of Latin names, a danger that must be avoided. In this paper, however, some of the F_1 hybrids between common American species are designated by binomials already in use, because culture experiments have shown these species and the F_1 hybrids to be almost identical.

INTERSPECIFIC F_1 HYBRIDS IN NATURE

Since most of the wild roses are highly heterozygotic for minor characteristics and do not breed true, it is not surprising that the F_1 cultures are also far from uniform. Many of them show some uniformity in habit and general appearance, except for an occasional aberrant individual. Minor characters, however, usually vary almost as much as in the wild cultures just analyzed.

Root-tip examinations of hybrids showed the expected number of chromosomes; that is, the sum of the haploid numbers of the parents. Apomixis has been found to occur very rarely in the roses of the Cinnamomeae. Many hundreds of buds have been shorn of everything but the hypanthium and achenes, just before they opened. No fruits have been obtained afterward, other than those originally reported for *R. blanda* (**14**).

The North American hexaploids are highly fertile and give fertile F_1 hybrids with each other. The F_1 from *R. acicularis*×*nutkana*, and the reciprocal, is of lower stature than *R. nutkana*. The stems are armed with scattered bristles and straight infrastipular prickles; the fruits are large (to 2 cm. in diameter) and usually pendant. Such plants in nature are often classified as *R. engelmannii* S. Wats., and this name is retained at present.

Rosa blanda gives fertile hybrids with *R. palustris*. Both species are diploids, and their ranges coincide over a large area in the region of the Great Lakes. Most of the F_1 hybrids resemble very closely

R. schuettiana Erlanson, a species that has been found in Wisconsin, Michigan, and New York (**13**). One out of 18 F_1 hybrids in one culture resembles *R. michiganensis* Erlanson, which type is also more rare in nature than *R. schuettiana*. *R. palustris* gave a sterile F_1 with *R. foliolosa*, although both are diploid.

R. blanda also gives fertile vigorous hybrids with the diploid *R. woodsii*. An intergrading series between these forms makes it hard to classify the diploid roses of the northern Great Plains region. In herbaria these forms are usually called *R. macounii*, a species that is probably a definite ecotype of *R. woodsii*. LUNELL's description of *R. naiadum* Lunell covers this hybrid, which resembles *R. woodsii* and may be used to designate intermediate forms. *R. woodsii* gave partially sterile F_1 hybrids with diploid *R. pisocarpa*.

R. blanda and *R. woodsii* both give partially sterile tetraploid F_1 hybrids with *R. acicularis*, which bear corymbs of flowers and fruits.[5] One of these crosses may have originally synchronized with mutations and given rise to the tetraploids of the Great Plains. *R. arkansana* gives a semisterile pentaploid F_1 with hexaploid *R. acicularis*.

The fertile tetraploid F_1 hybrid between *R. arkansana* (4x) and *R. carolina* (4x) is frequent on the eastern Great Plains and gives the species complex × *R. rudiuscula* Greene (**17**). The tetraploid species *R. carolina* and *R. virginiana* give a fertile F_1 which is intermediate and may be designated for convenience as × *R. obovata* Raf., since the type was collected in the Catskill Mountains, New York, where the parent species are both found. All triploid F_1 hybrids, from diploid crossed with tetraploid species, are of course highly sterile. The hypanthia usually all abort after anthesis, although hips and achenes have been observed on some plants of *R. blanda* × *virginiana*.

Although hybridization is thus known to be taking place in nature, it is nevertheless possible to recognize some of the F_1 types and also to distinguish a few definite Linnaean species. By allowing a wide range of parallel variation to these species, we can ignore later hybrid generations, in which the parent types probably segregate out again.

[5] The one large corymb recorded by CRÉPIN (**9**) for *R. acicularis* was on a plant collected by DRUMMOND at Cumberland House Fort. This specimen resembles *R. blanda* × *acicularis*.

Nomenclatural treatment of parallel variations

When I tabulated the combinations of characters in the collective species *R. acicularis* (12) and gave new varietal names to some of them, Mr. C. A. WEATHERBY wrote, "I am glad you are not afraid to provide names enough to cover all such combinations, when reasonably clear-cut." Since then it has been found that this is not practicable, for besides the individual variations which are shown in appendix I there are other variables. Stem colors—brown, green, and red—segregate; so do erect and decumbent, strict and subcernuous habits. Glaucous stems or foliage and non-glaucous are found in single cultures. Foliage texture and surface (lustrous or dull), color of filaments, petals and styles, all vary. Resistance to rusts and mildews differs in individuals of the same culture. We may easily have to deal with ten or twelve pairs of contrasting characters that are probably Mendelian, and usually assort independently. The resulting number of possible combinations is large, and hence a system of varietal names becomes unwieldy. Confusion lies this way also, for eventually we should be finding several varieties on the same bush, just as at present we find more than one species.

My firm conviction is that these minor parallel variations belong *sui generis* as to each Linnaean species, and that they can be treated only by using numerical tabulations, as has been done in the tables of appendix I. If any other method be followed, it leads to hopeless nomenclatural confusion, degradation of the species, and the sort of situation that exists in the American species of *Crataegus* (1).

Linneons in the American Cinnamomeae

As a result of these experiments and conclusions, I would admit only sixteen of the recognized groups of the Cinnamomeae in North America to unquestioned specific rank. Ten of them are collective species, namely: *R. acicularis*, *R. arkansana*, *R. blanda*, *R. californica*, *R. carolina*, *R. durandii*, *R. gymnocarpa*, *R. nutkana*, *R. pisocarpa*, and *R. woodsii*.

These groups all show a wide variation of habit types, possess geographic races within a wide range of distribution, and exhibit parallel variation. Once these phenomena are admitted, the species or Linneons can be distinguished from one another in the field, after

a little experience. They can often be identified from good herbarium material with the help of general characteristics and numerical variations. In table I, *R. blanda*, *R. californica*, *R. pisocarpa*, and *R. woodsii* are compared with regard to numerical variations of certain characteristics. In the accompanying analytical key (appendix III) an attempt has been made to employ and stress those characteristics which have been found not to occur as parallel variations. Chromosome number is an important characteristic and is given for each species in the key.

Species that are relatively stable and that can usually be recognized in the field and herbarium are: *R. foliolosa*, *R. nitida*, *R. palustris*, *R. rugosa*,[6] *R. spithamaea*, and *R. virginiana*.

Among these 16 species, five have a distribution that is chiefly eastern on this continent, nine are chiefly western, and only two, *R. arkansana* and *R. foliolosa*, are centrally distributed. This is interesting and logical when the geological history of the North American continent is taken into consideration.

Of the six F_1 hybrid species, only four (× *R. engelmannii*, × *R. michiganensis*, × *R. schuettiana*, and × *R. rudiuscula*) have been included in the key. × *R. obovata* and × *R. naiadum* closely resemble *R. carolina* and *R. woodsii* respectively.

Ecotype species

There are also seven groups that I now retain tentatively in specific rank which are suspected of being ecotypes (**40**) of various collective species.

R. williamsii Fernald, known only at Bic, Quebec, may be a calciphile ecotype of *R. blanda*.

R. macounii Greene, from Greene's description, resembles a compact form of *R. woodsii* with 7–11 small leaflets which I have obtained from Nebraska, South Dakota, and Saskatchewan. Greene

[6] A specimen of *R. rugosa* Thunb. (9645) from seed collected near Wrangell, Alaska, by Dr. A. S. Warthin in 1926 was raised at Ann Arbor. This is the more slender form with elliptical leaflets, which is given by Rehder (**32**) as *R. kamtchatica* Vent., a synonym of *R. rugosa*. More material of this species should be searched for in Alaska. This is the first record for an (apparently) endemic specimen of the *R. rugosa* group on the American continent.

says that his species "belongs to the region of dry elevated plains and is sub-alpine." LUNELL named a similar form *R. subnuda*.[7]

R. alcea Greene (syn. *R. subglauca* Rydb.) is a dwarf tetraploid which grows in the northern part of the range of *R. arkansana*, on the prairies of Canada. A plant that came from Craigmyle, Alberta, answering to the description of *R. alcea*, has grown at Ann Arbor (3492) for eight years and is only 2 dm. tall (fig. 19). It has never produced any flowering turions, but resembles *R. arkansana* in number of chromosomes, stamens, and teeth on the leaflets. It is suspected of being a northern ecotype of that species.

R. housei Erlanson, as already explained, appears to be an eastern swamp-inhabiting ecotype or ecophene of *R. arkansana*.

R. manca Greene (syn. *R. aciculata* Cockerell, *R. pecosensis* Cockerell) is a dwarf hexaploid of the southern Rocky Mountain region, closely allied to *R. nutkana*.

R. yainacensis Greene[8] (syn. *R. pinetorum* Heller, *R. myriadenia* Greene) is a low tetraploid form of dry habitats in the southern part of the range of *R. durandii* Crépin. It is more glabrous and has not been found with puberulent stems, but is judged to be closely related to, and perhaps a southern ecotype of, that species.

R. calvaria Greene is a diploid closely related to *R. gymnocarpa*, as GREENE also knew. The calyx is caudate attenuate and is not deciduous; RYDBERG therefore classified it with *R. pinetorum* (*R. yainacensis*), which, however, is tetraploid. Root tips from a plant of *R. calvaria* from Tuolumne Big Tree Grove, Tuolumne County, California, showed 14 somatic chromosomes. It is here retained as a questionable ecotype of *R. gymnocarpa* belonging to the southern Sierra Nevada. It is hoped that cultures will be grown in California to test this form,[9] as well as the interesting low growing tetraploids *R. yainacensis* and *R. spithamaea*.

[7] RYDBERG overlooked GREENE'S description and called pubescent variants of *R. woodsii* by this name. These were described as *R. grosseserrata* by NELSON, but NELSON'S name is not in my opinion synonymous with *R. macounii* or *R. subnuda*.

[8] Living material was obtained from Fort Ross, Calif., and from Pacific Grove, Monterey Bay, the type locality of *R. pinetorum*. Herbarium material shows that *R. yainacensis* and *R. pinetorum* are essentially the same, and the former name has priority.

[9] *R. bolanderi* Greene and *R. covillei* Greene were also reported by GREENE to belong to the group of *R. gymnocarpa* and to possess short ovate non-deciduous sepals. This characteristic is not sufficient for specific distinction from *R. gymnocarpa*, and these two "species" are considered to be merely minor variants.

The octoploid form of *R. acicularis* is probably the type form since a hexaploid has not been reported for Europe. CRÉPIN (**10**) suggested that the American form be separated as *R. sayi* Schw. On the evidence of flowering time and stamen number (table II; fig. 2), it would be justifiable to call the hexaploid *R. acicularis* var. *sayi* (Schw.) Rehder, at least for horticultural use. The two forms cannot be distinguished in herbarium material and the distribution of the octoploid needs to be worked out.

In appendix II there are listed alphabetically the 16 Linnaean species that are now retained, together with the ecotype species, if any, and synonyms related to each from the evidence of parallel variation. The synonyms include the 110 species given by RYDBERG (**34**) for the sections Carolinae, Cinnamomeae, and Gymnocarpae, plus five species listed by him as synonyms for *R. macounii*, plus three species described by the writer (**13**). A brief preliminary account of these conclusions was previously published (**20**).

Summary

1. Herbarium, field, and garden studies upon extensive series of North American wild roses belonging to the section Cinnamomeae have shown that many of the characteristics commonly used to distinguish between species are individual variations. These unreliable characteristics may occur combined in every possible way, sometimes in plants of a single culture. They also appear in every species and give good series of parallel variations. Because in the past rhodologists have been inclined to give these minor variations and their multitudinous combinations specific or varietal rank, nomenclatural confusion has resulted.

2. The various diagnostic characteristics are discussed and evaluated chiefly from evidence obtained from growing cultures of plants raised from the seeds of single wild individuals. As many as ten so-called species have appeared in one culture.

3. The offspring of isolated diploid plants of *R. woodsii* from the Great Basin of Utah showed as much variability and pollen sterility as offspring of plants of *R. blanda* from northern Michigan, which grew close to roses belonging to other species groups. Hybridization is not therefore the most important source of variation.

4. Tables are presented which demonstrate the variation in minor

characteristics in cultures of the groups of *R. blanda*, *R. californica*, *R. pisocarpa*, and *R. woodsii*.

5. On the evidence of data from these and other cultures, the sections Carolinae and Gymnocarpae have been merged with the section Cinnamomeae. One hundred ten species in this section given by RYDBERG (**34**) and three species described by the writer have been placed in 16 Linnaean species. These 16 species are relatively stable (although individuals are highly heterozygous) and they can be distinguished from one another by general, morphological, numerical, cytological, and physiological characteristics. Ten of the sixteen are collective species with wide ranges of distribution.

6. Seven other species are suspected of being ecotypes of the various collective species but have been retained in specific rank.

7. Interspecific F_1 hybrids have been raised after crossing species with ranges that overlap. Several of these hybrids are fertile and resemble roses that occur in nature. Six previously described species are considered on this evidence to be F_1 hybrids.

8. These results which attribute a few highly variable Linnaean species to the genus *Rosa* agree with those obtained by BOULENGER from data from European rose species.

9. Sterility arises from (a) interchange, (b) internal polyploidy, (c) hybridization with polyploid species. Polyploidy is therefore an obstacle to the fusion of species.

10. Ninety-one specific names, chiefly from RYDBERG (**34**), have been given as synonyms in an alphabetical list of Linneons. An analytical key is appended which includes 16 Linnaean species, seven ecotype species, and four hybrid species in the section Cinnamomeae.

UNIVERSITY OF MICHIGAN
ANN ARBOR, MICHIGAN

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Appendix I

Key to symbols used for parallel variations in cultures

B Bristles
- o absent
- 1 basal
- 2 to twig tips
- 3 deflexed
- 4 glandular
- 5 pubescent

P Prickles
- o absent
- 1 straight
- 2 terete
- 3 deflexed
- 4 flattened
- 5 curved
- 6 ascending

L Leaflet shape
- 1 oval
- 2 elliptical
- 3 ovate
- 4 obovate
- 5 cordate base
- 6 orbicular

Lsc Leaflet surface covering
- o glabrous
- 1 appressed pubescent, both surfaces
- 1v villous, both surfaces
- 2 appressed pubescent beneath
- 2v villous beneath
- 3 pubescent on veins beneath
- 3v villous on veins beneath
- 4 gland-muriculate
- 5 gland-muriculate on veins below
- 6 gland-pruinose
- 7 glandular-granuliferous

R Rhachis
- o glabrous
- 1 pubescent
- 2 gland-pruinose
- 3 gland-hispid
- 4 setaceous

T Teeth on leaflets
- 1 simple
- 2 bidentate
- 3 serrulate
- 4 gland-serrulate

H Hypanthium in fruit
- o glabrous
- 1 globose
- 2 depressed globose
- 3 with neck (urceolate)
- 4 pyriform
- 5 ellipsoid
- 6 pilose
- 7 gland-hispid
- 8 hispid

S Sepals
- o glabrous
- 1 puberulent on edge
- 2 puberulent on back
- 3 gland-hispid on edge
- 4 gland-hispid on back
- 5 reflexed
- 6 spreading
- 7 erect
- 8 connivent
- 9 deciduous

F Pedicel
- o glabrous
- 1 puberulent
- 2 pilose
- 3 gland-hispid
- 4 glandular-granuliferous

VARIATION IN OFFSPRING OF SINGLE WILD INDIVIDUALS

TABLE A

COLLECTIVE SPECIES R. BLANDA

CULTURE 3753/7 (56 OFFSPRING*). PARENT: R. BLANDA VAR. GLANDULOSA, MACKINAC CO., MICHIGAN. HIPS ALL GLABROUS AND SMOOTH

B	P	L	Lsc	R	T	H	S	IDENTIFICATION ACCORDING TO RYDBERG'S KEY
Parent								
1	0	1	12v	1	1	5	47	blanda var. glandulosa
Offspring								
1	0	1	12v	1	4	5	47	acicularioides
2	0	1	12v	1	1	1	47	blanda var. hispida
1	0	1–4	12v	1	1	1	37	blanda
1	0	1	12v	1	1	1	46	blanda
2	0	1–2	12v	4	1	1	46	blanda
1	0	1	12v	1	1	1	47	blanda
1	0	1–2	12v	1	1	1	47	blanda
1	0	1	12v	1	1	4	07	blanda var. glandulosa
1	0	1	1	1	1	3–4	47	blanda var. glandulosa
1	0	1	12v	4	1	1–5	47	blanda var. glandulosa
2	0	1–2	2	1	1	1–4	07	blanda var. glandulosa
2	0	1–4	2	1	1	.....	.....	Dwarf
2	0	1	12v	1	1	1–4	37	blanda var. glandulosa
1	0	1	2	4	1	1	46–7	blanda
1	0	1	12v	1	1	1–4	36	blanda
1	0	1–4	12v	1	1	1–4	46	blanda
1	0	1	12v	1	1	1	46–7	blanda
2	0	1–4	2	1	1	.....	.....	Dwarf
1	0	1–2	12v	1	1	1–2	06	blanda
1	0	1–4	2	4	1	1	47	blanda
2	0	1	12v	1	2–4	2	07	acicularioides
1	0	1	12v	1	1	1	07	blanda
1	0	1–2	2	1	1	1	36	blanda
1	0	1	12v	4	1–2	1	47	blanda
1	0	1–4	12v	1	1	1–4	47	blanda
1	0	1	1	1	1–2	1	46	blanda
1	0	1	1	1	1	1–2	47	blanda
1	0	1	12v	4	1	1	47	blanda
1	0	1	1	1	1	3	06–5	blanda var. glandulosa
1	0	1	12v	1	1	3	35	blanda var. glandulosa
1	0	1	12v	1	1	1	47	blanda
1	0	1–2	2	1	1	2	47	blanda
2	0	1–4	12v	1	1–4	1	46	acicularioides
1	0	1	2	1	1	1	37	blanda
1	0	1	2	1	1	1	36	blanda
1	0	1–2	12v	4	1	1	47	blanda
2	0	3–6	2	4	1	.....	.....	Dwarf
1	0	1–2	12v	1	1	5	47	blanda var. glandulosa
1	0	1	12v	1	1	4	47	blanda var. glandulosa
1	0	1	1	1	1–4	1	07	blanda
2	0	1–4	2	1	1	.....	3	blanda (sterile triploid)
2	0	1	2	4	1	1	47	blanda
2	0	1–4	2	1	1	4	46	blanda var. glandulosa

TABLE A—*Continued*

B	P	L	Lsc	R	T	H	S	Identification according to Rydberg's key
Offspring —*Continued*								
1	0	1	12V	1	1	1	47	blanda
1	12	1	12V	1	1	4	37	blanda var. glandulosa
1	0	1–2	3	1	1	2	36	blanda
1	0	1	12V	1	2–4	1	07	blanda
1	0	1	0	1	1	2	47	subblanda
2	0	1–4	2	4	1	5	46	blanda var. glandulosa
2	0	1–4	12V	1	1–4	.....	.....	Dwarf
1	0	1	12V	1	1	2	46	blanda
2	0	1	12V	1	1	1	07–6	blanda var. hispida
2	0	2	0	4	1	.....	.....	subblanda (dwarf)
1	2–5	1	12V	1	1	1	07	palustriformis
2	0	1–2	12V	1	1	1	07	Sterile tetraploid dwarf

* Stem color: reddish brown, 46; greenish brown, 10.

TABLE B

COLLECTIVE SPECIES R. BLANDA (CONTINUED)

CULTURE 12325 (10 OFFSPRING). PARENT: R. BLANDA, GASPÉ PENINSULA, QUEBEC. ALL PLANTS WITHOUT INFRASTIPULAR PRICKLES AND WITH 5–9 OVAL LEAFLETS AND SIMPLE TEETH. FORMULAE GIVEN FOR OFFSPRING ONLY

B	Lsc	R	H	S	Identification
1	1	1	1–27	46–7	blanda var. carpohispida
2	1	3	20	36–7	blanda
1	1	3	10–7	47	blanda var. carpohispida
1	0	0	10	37	subblanda
1	1	3	1–47	46–7	blanda var. carpohispida
1	17	1	10	46–7	blanda
1	2	1	1–50	06–7	blanda var. glandulosa
2	1	3	10	47	blanda
1	0	0	10	36–7	subblanda
1	1	1	27	07	blanda var. carpohispida

TABLE C

COLLECTIVE SPECIES R. WOODSII*

CULTURES 12191 (9 OFFSPRING) AND 12193 (1 OFFSPRING). PARENT: R. MACOUNII, SALT LAKE CITY, UTAH. ALL PLANTS BRISTLY AT BASE, AND WITH GLOBOSE GLABROUS HIPS

P	L	Lsc	R	T	H	S	IDENTIFICATION
12191							
Parent							
12	1–2	1	1	1	2	17	macounii
Offspring							
32	1	1	1	1	1	17	puberulenta
0	1	1	1	1	1	.....	Dwarf
12	1	0	0	1–2	1	17	woodsii
32	3–2	17	1	1–2	1	07	puberulenta
45	1–3	0	14	1	1	27	woodsii
12	1–4	17	1	1	2	07	macounii
0	4–2	17	1	1	1	27	salicetorum
12	4–2	17	1	4	2	27	fendleri
32	1–2	1	1	3–4	1	17	granulifera
12193							
Parent							
12	1–2	1	1	1	1	06	macounii
Offspring							
15	1–4	18	12	1	1	07	puberulenta

* Culture 12211 (9 offspring). Parent: *R. macounii*, Reno, Nevada. Height of offspring: over 1 m., 7 plants; dwarfs (under 0.5 m.), 2 plants. Badly rusted, 3 plants. Stems: green in 5 plants, brown in 1 plant, red in 4 plants, glaucous in 2 plants. According to RYDBERG's key the plants would be classified from leaf characters as: *R. fendleri*, 3 plants; *R. macounii*, 6 plants. Only 2 flowered in 1931 and both had 50% of the pollen shriveled.

Culture 12220 (38 offspring). Parent: *R. pyrifera*, Reno, Nevada. Height: about 1 m., 33 plants; less than 5 dm., 5 plants. Stem color: dull brown, 36 plants; red, 2 plants; glaucous, 15 plants; badly rusted, 9 plants. According to leaf characters, by RYDBERG's key the offspring would be classified as: *R. macounii*, 32 plants; *R. fendleri*, 5 plants; *R. hypoleuca*, 1 plant; *R. fendleri*, 1 plant.

TABLE D

COLLECTIVE SPECIES R. WOODSII

CULTURE 12205 (21 OFFSPRING). PARENT: R. MACOUNII, SOUTH OF SPARKS, WASHOE COUNTY, NEVADA. PARENT 2.5 M. TALL. ALL PLANTS WITH BRISTLES AT BASE AND GLABROUS HIPS

B	P	L	Lsc	T	H	S	IDENTIFICATION
Parent							
1	12	2–3	17	1–2	5	16	macounii
Offspring							
1	12	1–2	17	1–3–4	3	05	macounii (semisterile with multivalents 2n=14)
1	12	1–2	17	1	4	25	arizonica (?)
1	12	1–2	17	1–2	.....	.....	.....
1	14	1	1	1	1–4	17	macounii (semisterile with multivalents 2n=14)
1	12	3	0	1–2	.....	.....	Dwarf
1	16	2	27	1	5	16	macounii (semisterile)
2	0	2–4	17	1–2	5	06	macounii (semisterile)
1	12	1–2	27	1–3–4	5	17	fendleri
1	12	2	17	1–3	1	25	fendleri
2	0	1–2	1	1	.....	.....	Dwarf, badly rusted
2	1	1	17	1–2–3	1	05	fendleri
1	12	1	1	1	1	06	macounii
2	0	1–4	17	1	1	16	macounii
2	0	2	17	1–3–4	.....	.....	fendleri
2	12	16	17	1	.....	0	macounii (2n=21, sterile)
1	12	1–3	1	1	1	15	macounii
2	12	1–3	17	1–3–4	1	15	fendleri
2	12	1	16	1–4	.....	.....	fendleri
1	12	16	06	1–2	1	07	hypoleuca
1	12	2–4	17	1	35	17	standleyi
2	12	1–2	36	1–4	1	17	hypoleuca

TABLE E

COLLECTIVE SPECIES R. WOODSII

CULTURE 12207 (11 OFFSPRING*). PARENT: R. FENDLERI, SOUTH OF SPARKS, WASHOE CO., NEVADA. ALL PLANTS 1–1.5 M. WITH WEAK SCATTERED BRISTLES AND FEW TERETE PAIRED PRICKLES

L	Lcs	R	T	H	S	IDENTIFICATION
Parent						
1	17	12	1–4	1	46	fendleri
Offspring						
1	07	0	1–4	.....	.....	hypoleuca
1	17	12	1–4	.....	.....	fendleri
2	16	12	1	5	15	fendleri
2	16	12	1	.....	.....	fendleri
1	36	12	1–4	1	236	fendleri (resin-scented)
1	0	2	1	1	06	woodsii
1–3	0	2	1–3	1–5	05–6	woodsii
1	0	2	1–3	1	25	woodsii
1	0	2	1	1	16	woodsii
1	0	2	1	.....	.....	woodsii
6	0	2	1	.....	.....	woodsii

* Highly homozygous for this group. Pollen sterility low (5–12%) in four plants (see table III).

TABLE F

COLLECTIVE SPECIES R. WOODSII

CULTURE 12209 (52 OFFSPRING). PARENT: R. PYRIFERA, SPARKS, WASHOE CO., NEVADA. OFFSPRING 0.3–2 M. HIGH WITH STEMS BRISTLY AT BASE, SCATTERED BRISTLES AND WEAK PAIRED PRICKLES ON TWIGS. STEMS REDDISH. LEAFLETS BROADLY OVAL, YELLOWISH GREEN. MANY RUSTED. FEW FLOWERS; SEPALS ERECT TO REFLEXED

B	P	Lsc	R	T	H	S	IDENTIFICATION
Parent							
1	12	3	2	1	4–5	1	pyrifera
Offspring							
1	12	0	0	1	.....	.....	woodsii
1	12	06	2	1–4	1	15	hypoleuca
1	12	26	2	1	.....	.....	fendleri
1	12	26	2	1	.....	.....	fendleri (rusted)
1	12	27	2	1	.....	.....	.....
2	0	07	2	1	.....	.....	Dwarf (15 cm.)
2	0	27	2	1	.....	.....	Dwarf (8 dm.)
2	12	2	1	1	1	045	gratissima (?)
1	12	0	1	1	.....	.....	woodsii
1	13	2	4	1	.....	.....	macounii
1	13	2	4	1	.....	.....	macounii
2	0	26	2	1	.....	.....	fendleri (dwarf, 8 dm.)
2	12	26	2	1	1	06–7	gratissima
2	13	27	4	1–2	13	14	gratissima (2n=14, sterile)
1	0	1	1	1	.....	.....	.....
2	12	3	1	1	.....	.....	woodsii
2	12	27	2	1	1	15	macounii
1	12	0	2	1	.....	.....	woodsii
1	12	2	1	1	.....	.....	macounii
2	0	0	2	1	.....	.....	woodsii
2	0	0	2	1	.....	.....	gratissima
2	12	27	2	1	5	0	pyrifera
2	12	06	2	2–4	.....	.....	hypoleuca
2	0	06	2	2–4	.....	.....	hypoleuca (rusted)
2	12	0	0	1	.....	.....	woodsii (rusted)
2	12	2	1	1	4	0	pyrifera
2	0	2	1	1	.....	.....	.....
2	12	2	1	1	.....	.....	.....
2	12	07	2	1	.....	.....	hypoleuca
2	12	2	1	1	.....	.....	.....
2	0	2	1	1	.....	.....	.....
2	0	2	1	1	1	146	gratissima
2	12	0	0	1–4	.....	.....	hypoleuca
2	0	2	1	1	.....	.....	.....(badly rusted)
2	12	17	2	1	.....	.....	fendleri (rusted)
2	12	17	2	1	.....	.....	fendleri
2	12	07	2	1	.....	.....	hypoleuca
2	12	2	1	1	.....	.....	gratissima (badly rusted)
2	0	27	2	1	.....	.....	gratissima
1	12	27	2	1	.....	.....	gratissima

TABLE F—*Continued*

B	P	Lsc	R	T	H	S	Identification
2	0	0	2	4	.....	.....	hypoleuca (rusted)
2	0	27	2	1	.....	.....	gratissima
2	0	27	2	1–2	3	2	gratissima
2	12	27	2	1	.....	.....	.....
2	0	27	2	1	.....	.....	.....
2	0	0	2	1	.....	.....	woodsii (rusted)
2	0	2	2	4	.....	.....	fendleri (rusted)
2	0	27	2	1	.....	.....	gratissima
2	0	27	2	1	.....	.....	2n=21
2	12	27	2	1	1	2	gratissima
2	0	0	0	1	.....	.....	woodsii
2	12	0	0	1	.....	.....	woodsii

TABLE G

COLLECTIVE SPECIES R. WOODSII

CULTURE 12212 (2 OFFSPRING). PARENT: R. PUBERULENTA, RENO, NEVADA

B	P	L	Lsc	T	H	S	Identification
Parent							
1	13	1	2	1	1–4	27	puberulenta
Offspring							
2	0	1–3	1	1	.....	.....	macounii
13	13	1–2	27	1	4	27	neomexicana (5 ft. tall; bad pollen, 3%)

TABLE H

COLLECTIVE SPECIES R. WOODSII

CULTURES 12213 (4 OFFSPRING) AND 12218 (6 OFFSPRING). PARENT: R. SALICETORUM, RENO, NEVADA

B	P	L	Lsc	T	H	S	IDENTIFICATION
12213							
Parent							
1	0	1	17	1	4	17	salicetorum
Offspring							
2	0	1–2	17	1	.....	.....	macounii (rusted)
1	0	2	17	1	1	15	salicetorum (rusted)
2	0	2	27	1	1	16	macounii
2	12	1–2	27	1–2	.....	.....	macounii
12218							
Parent							
1	0	1	17	1	1	1	salicetorum
Offspring							
2	0	1	07	1–4	.....	.....	hypoleuca
2	0	1	17	1–2–4	.....	.....	fendleri
2	0	1	1	1	.....	.....	macounii
2	0	2	17	1–2	.....	.....	fendleri
2	0	1	17	1	.....	.....	macounii
2	0	1	17	3	.....	.....	macounii

TABLE I

COLLECTIVE SPECIES R. WOODSII

CULTURE 12220 (38 LIVING OFFSPRING,* DATA IN TABLE ONLY FOR 12 THAT FLOWERED). PARENT: R. PYRIFERA, RENO, NEVADA. ALL PLANTS WITH BRISTLES AT BASE AND STRAIGHT TERETE PRICKLES

L	Lsc	T	H	S	IDENTIFICATION
Parent					
1	2	1	4	17	pyrifera
Offspring					
1	17	1	4	17	pyrifera
1	26	1–4	1	17	fendleri
1	2	1	1	17	macounii
1	27	1	1	16	macounii
1–2	2	1	1	17	macounii
1	27	1	4	17	macounii
1	06	1–4	1	17	hypoleuca
1	2	1	1	17	macounii
1	37	1	1	16	macounii
1	27	1	1	17	macounii
1	2	1	1	16	macounii
1–2	2	1	1	16	macounii

* General segregation of characters among 38 plants. Height: about 1 m., 33 plants; about 0.5 m., 5 plants. Badly rusted, 9. Stem color: dull brown, 36; reddish, 2; glaucous stems, 15. Leaf types: "*R. macounii*," 32 plants; "*R. woodsii*," 1; "*R. fendleri*," 1; "*R. hypoleuca*," 1.

TABLE J

COLLECTIVE SPECIES R. WOODSII

CULTURE 12231 (3 OFFSPRING). PARENT: R. GRANULIFERA, PYRAMID LAKE, NEVADA*

B	P	L	Lsc	T	H	S	IDENTIFICATION
Parent							
23	25	1	26	4	1	17	granulifera
Offspring							
2	12	1–2	37	1	.....	.....	gratissima
23	23	2–3	27	1–4	1	16	granulifera
2	12	1	27	1	.....	.....	gratissima

* Parent plant isolated in sagebrush plains and desert. No other roses for 40 miles around. All 3 plants rusted.

TABLE K

COLLECTIVE SPECIES R. PISOCARPA

CULTURE 12248 (4 OFFSPRING*). PARENT: R. PISOCARPA, MT. SHASTA CITY, CALIF. ALL PLANTS: HABIT STRICT, HEIGHT 1–1.5 M; A FEW BRISTLES AT BASE AND A FEW WEAK, TERETE INFRASTIPULAR PRICKLES; HYPANTHIUM GLABROUS AND GLOBOSE, 7–9 MM. DIAM.

L	Lsc	T	S	IDENTIFICATION
Parent				
1–3	0	1	4	pisocarpa
Offspring				
1–3	1	1	.....	.....
2	3	1	15	ultramontana
1–3	17	1	15	ultramontana
1–3	2	1	46	pisocarpa

* Stems: green, 2; reddish, 2. All plants rusted.

TABLE L

COLLECTIVE SPECIES R. PISOCARPA

CULTURE 12259 (1 OFFSPRING). PARENT: R. PRINGLEI, JACKSONVILLE, OREGON

B	P	L	Lsc	T	H	S	IDENTIFICATION
Parent							
o	o	1–3	3	1	1–5	37	pringlei
Offspring							
o	o	1–2–3	2	1	.....	3	anacantha (triploid)

TABLE M

COLLECTIVE SPECIES R. PISOCARPA

CULTURES 12262 (1 OFFSPRING) AND 12268 (1 OFFSPRING). PARENT: R. COPELANDII, ASHLAND CAÑON, OREGON

B	P	L	Lsc	T	H	S	IDENTIFICATION
12262							
Parent							
1	12	3	o	1	5	37	copelandii
Offspring							
2	12	3	3	2–4	.....	.....	.....
12268							
Parent							
2	1	3	o	1	5	37	copelandii
Offspring							
o	o	2–3	o	1	3	37	copelandii

TABLE N

COLLECTIVE SPECIES R. PISOCARPA

CULTURE 12280 (25 OFFSPRING,* DATA IN TABLE ONLY FOR 13 THAT FLOWERED); PARENT: R. PISOCARPA, CORVALLIS, OREGON. ALL STEMS GREENISH, GLAUCOUS. ALL LEAFLETS GLAUCOUS BELOW

B	P	L	Lsc	T	H	S	IDENTIFICATION
Parent							
o	26	35	2	1	1	16	pisocarpa
Offspring							
o	o	35	2	1	1	47	anacantha
o	o	1	2	1	1	46–9†	anacantha
1	o	35	2	1	1	46	pisocarpa
1	o	35	2	1	1	47	pisocarpa
1	o	1–35	3	1	1	10–46	pisocarpa‡
o	26	35	3	1	3	10–3	pringlei‡
1	o	35	3	1	1	47	pisocarpa
1	o	35	3	1	1	36–9†	pisocarpa
o	26	1–35	3	1	1	36	pisocarpa
1	o	35	2	1	1	47	pisocarpa
o	26	15	2	1	3	46	pringlei
o	o–26	35	2	1	1	47	pisocarpa
o	26	35	2	1	1	47	pisocarpa

* Bristles: none, 17; basal, 8. Prickles: none, 20; terete and ascending, 5. Stems: slender, with arched branches, except 3 plants. One plant leaflets orbicular, cordate at base (22×20 mm.; 11×10 mm.).

† Note partially deciduous sepals.

‡ RYDBERG gives eglandular sepals to *R. pringlei* and *R. copelandii*, and insists upon glandular-hispid sepals for *R. pisocarpa*.

TABLE O

COLLECTIVE SPECIES R. PISOCARPA

CULTURE 12284 (21 OFFSPRING,* DATA IN TABLE ONLY FOR 9 THAT FLOWERED). PARENT: R. PRINGLEI, SOUTH OF CORVALLIS, OREGON. RARELY A FEW BRISTLES AT BASE

B	P	L	Lsc	T	H	S	IDENTIFICATION
Parent							
o	o–26	3	2	1	35	46	pringlei
Offspring							
o	26	3	2	1	1	47	pisocarpa
1	26	3	2	1	13	46	"
1	26	3	2	1	1	47	"
1	26	1	3	1	13	45–6	"
o	26	3	2	1	1	47	"
1	26	1	2	1	13	45	"
o	26	3	2	1	1	47	"
o	o	3	2	1	13	47	anacantha
1	26	3	3	1	1	47	pisocarpa

* Stems 0.9–2 m., 1 dwarf (4 dm.); glaucous red-brown (18 plants), greenish (3 plants); arched (18 plants), strict (3 plants); few slender ascending paired prickles (19 plants), completely unarmed (2 plants). Leaflets ovate to oval, base usually somewhat cordate, pale glaucous and finely appressed puberulent beneath. Rachis gland-hispid in 2 plants. Formula of dwarf, BO P26 Lsl Lcs2 T4.

TABLE P

COLLECTIVE SPECIES R. CALIFORNICA

CULTURE 12295 (4 OFFSPRING*). PARENT: R. CALIFORNICA, CASHION CAÑON, CONTRA COSTA CO., CALIF.

B	P	L	Lsc	T	H	S	F	IDENTIFICATION
Parent								
1	34	13	IV	1	13	2	2	californica
Offspring								
1	0	13	IV7	1	1	46	2	johnstonii
1	14	23	IV	1	4	26	2	johnstonii
2	0	13	IV	1	.....	.....	.....	Dwarf
1	12	23	IV	1	4	26–7	2	johnstonii

* Habit: arched, 3; strict, 1; dwarfs, 1. Stems: brown, 3; green, 1; glaucous, 1.

TABLE Q

COLLECTIVE SPECIES R. CALIFORNICA

CULTURE 12303 (5 OFFSPRING*). PARENT: R. CALIFORNICA, BERKELEY, CALIF. STEMS ALL RED-BROWN. PRICKLES VARIABLE EVEN ON SAME BUSH. HYPANTHIA AND PEDICELS PILOSE

B	P	L	Lsc	T	H	S	IDENTIFICATION
Parent							
1	25	1	IV2	1	1	17	californica
Offspring							
2	23–5	1–6	2V7	1	1	27	californica
2	23–5	1–6	2V7	1	1	23	californica
1	12	1–6	IV	1	.....	.....	johnstonii
2	16	1	IV	4	.....	.....	bidenticulata (dwarf)
2	14	1	IV7	1	1	26	johnstonii

* Habit: arched, 3; strict, 1; dwarfs, 1.

TABLE R

COLLECTIVE SPECIES R. CALIFORNICA

CULTURE 12307 (44 OFFSPRING). PARENT: R. SANCTAE-CRUCIS, REDHILL, CALIF. COARSE STURDY PLANTS. STEMS 1–1.5 M., MUCH BRANCHED, ARCHING TO GROUND. LEAFLETS 5–7 (RARELY 9). FLOWERS CORYMBOSE. STIGMATIC HEADS FLAT TO 3 MM. HIGH

B	P	L	Lsc	T	H	S	F	IDENTIFICATION
Parent								
1	14	1–3	IV7	1–4	13	25	2	sanctae-crucis
Offspring								
1	45	1–3	IV7	1	2	16	0	brachycarpa (stigmatic head raised 3 mm.)
2	1–32	1–2	IV	1	1	25	2	johnstonii
1	12	1–6	IV	1	1	26	24	johnstonii
1	1–34	1–4	IV	1	3	26	2	johnstonii
1	14	1–3	IV	1	1	25	23	davyi
2	12	1–3	IV7	3–4	1	27	2	?corymbiflora (resin-scented)
2	12	1	IV7	1–4	1	27	2	?corymbiflora
1	2–43	1	IV7	1–4	1	237	3	breweri (stem under flowers glandular-hispid)
2	21	1–3	IV	1	1	2	2	johnstonii
2	1–32	1–2	IV	1	1	25	2	johnstonii
1	45	1–3	IV	1	1	2	2	californica
1	14	1–6	IV	1	1	2	13	davyi
2	12	1–4	IV	1	1	26	2	johnstonii
1	45	1–3	IV7	1	2	16	0	californica
1	14	1–6	IV7	1	1	247	3	sanctae-crucis
2	12	1	IV7	4	1	247	3	corymbiflora
2	12	4–6	IV	1	1	27	0	johnstonii
2	12	1	IV	1	3	27	0	johnstonii
1	12–4	4–6	IV	1	1	2	23	johnstonii
2	12–4	1	17	1	1	27	0	johnstonii
1	12–4	1–2	IV	1	1	2	2	johnstonii
1	45	1	IV7	1	1	2	2	brachycarpa (stigmatic head raised 3 mm.)
2	12	1	27	2–4	1	24	23	?corymbiflora (dwarf 4 dm.)
2	12	1	IV	1	1	2	0	johnstonii
1	12	1–4	IV	1	13	27	0	johnstonii
1	12	1	IV	1	1	527	0	johnstonii (stem under flowers glandular)
2	12	1–3	IV	1	1	2	0	johnstonii
1	14	1	IV7	1	1	27	2	sanctae-crucis
1	12–3	1	IV7	1	1	2	2	johnstonii (stem under flowers pilose)
1	14	1–3	IV	1	1	27	2	davyi
2	12	1–6	IV7	1–4	1	26	2	bidenticulata
2	12	1	27	1	1	2	0	johnstonii

TABLE R—*Continued*

B	P	L	Lsc	T	H	S	F	Identification
Offspring —*Cont.*								
1	12	1–4	IV	1	1	2	o	johnstonii
1	45	1–3	IV	1	13	26	o	californica
1	14	1	IV7	1	1	27	2	sanctae-crucis
1	14	1–6	27	1	1	2	2	sanctae-crucis
1	45	1	IV7	1–4	13	26	2	aldersonii (stem under flowers pilose)
1	45	1	IV	1	13	27	o	californica
2	12	1	1	1	.....	.....	.....	johnstonii (stems weak, 1 m.)
1	12	1–4	IV	1	1	2	2	johnstonii
1	12	1	IV	1	1	2	2	johnstonii
1	12	1	IV	1	1	2	2	johnstonii
1	45	1–5	IV	1	1	26	2	californica
1	43	1–2	IV7	1–2	14	27	4	greenei

TABLE S

COLLECTIVE SPECIES R. CALIFORNICA

CULTURE 12314 (3 OFFSPRING). PARENT: R. BIDENTICULATA, HESPERIA, CALIF. STEMS STRICT; FLOWERS 1–3

B	P	L	Lsc	T	H	S	F	Identification
Parent								
1	12	1–3	3	1–2	1	26	o	bidenticulata
Offspring								
2	12	1–6	07	1–4	1	05	o	bidenticulata
2	12	1–3	3	1	1	06	o	.....
2	12	1–6	27	1–4	1	06	2	bidenticulata (rachis and stipules glandular)

Appendix II

An alphabetical list of the 16 Linnaean species in the section Cinnamomeae that are considered worthy of specific rank, together with six F_1 hybrid species, follows. Seven ecotype species are placed under their related Linneons.

The rose species given in the North American Flora by RYDBERG (**34**), also by the writer in Rhodora (**13**), in the sections Carolinae, Cinnamomeae, and Gymnocarpae, are grouped alphabetically under the Linneon in which each is now included. For further synonymy the North American Flora should be consulted.*

R. acicularis Lindl. 1820. 2n = 56 or 42 (two races)

Synonyms: *bourgeauiana Crépin, butleri Rydb., collaris Rydb.*

R. arkansana Porter, 1874. 2n = 28

Ecotype species: R. alcea Greene, R. housei Erlanson

Syn.: *R. lunellii Greene* (= R. alcea), *R. polyanthema Lunell, ratonensis Erlanson, subglauca Rydb.* (= R. alcea), *suffulta Greene*

R. blanda Ait. 1789. 2n = 14

Ecotype species: R. williamsii Fernald

Syn.: *acicularioides Schuette, johannensis Fernald, palustriformis Rydb., subblanda Rydb.*

R. californica Schlecht. & Cham. 1827. 2n = 28

Syn.: *R. aldersonii Greene, bidenticulata Rydb.* (= R. californica var. bidenticulata (Rydb.) comb. nov.), *brachycarpa Rydb., corymbiflora Rydb., davyi Rydb., delitescens Greene, greenei Rydb., johnstonii Rydb., myriantha Carr., pilifera Rydb., sanctae-crucis Rydb.*

R. carolina L. 1753. 2n = 28

Syn.: *deamii Erlanson, gemella Willd., lyoni Pursh, nanella Rydb., palmeri Rydb., petiolata Rydb., serrulata Raf., texarkana Rydb., treleasii Rydb.*

R. durandii Crépin, 1875. 2n = 28

Ecotype species: R. yainacensis Greene

Syn.: *muriculata Greene* (in part), *myriadenia Greene* (= R. yainacensis), *pinetorum Heller* (= R. yainacensis)

×R. engelmannii S. Wats. 1889. 2n = 42

R. foliolosa Nutt. 1840. 2n = 14

* Synonyms given by RYDBERG are not included in this list, except those he gave for *R. macounii Greene*, because culture experiments show that one collective species (*R. woodsii*) and an ecotype of the same species (*R. macounii*) are mingled in his designation of *R. macounii*.

R. gymnocarpa Nutt. 1840. 2n = 14
Ecotype species: R. calvaria Greene
Syn.: *bolanderi Greene, bridgesii Crépin, covillei Greene, dasypoda Greene, leucopsis Greene, oligocarpa Greene, prionota Greene*
×R. michiganensis Erlanson, 1928. 2n = 14
×R. naiadum Lunell, 1913. 2n = 14
R. nitida Willd. 1809. 2n = 14
R. nutkana Presl, 1851. 2n = 42
Ecotype sp.: R. manca Greene
Syn. *aleutensis Crépin,* aciculata Ckll.* (= R. manca), *brownii Rydb., columbiana Rydb., macdougali Holz., melina Greene, muriculata Greene* (in part), *oreophila Rydb., pecosensis Ckll.* (= R. manca), *spaldingii Crépin, underwoodii Rydb.*
×R. obovata Raf. 1820. 2n = 28
R. palustris Marsh. 1785. 2n = 14
Syn.: *dasystoma Rydb., floridana Rydb., lancifolia Small, obtusiuscula Rydb.*
R. pisocarpa A. Gray, 1872. 2n = 14
Syn.: *anacantha Greene, copelandii Greene, eastwoodiae Rydb., pringlei Rydb., rivalis Eastw., rotundata Rydb., ultramontana (S. Wats.) Heller*
×R. rudiuscula Greene, 1911. 2n = 28
Syn.: *aucuparia Rydb., bushii Rydb., conjuncta Rydb., relicta Erlanson*
R. rugosa Thunb. 1784. 2n = 14
×R. schuettiana Erlanson, 1928. 2n = 14
R. spithamaea S. Wats. 1880. 2n = ?
Syn.: *adenocarpa Greene, dudleyi Rydb., granulata Greene, sonomensis Greene*
R. virginiana Mill. 1768. 2n = 28
Syn.: *R. bicknellii Rydb.*
R. woodsii Lindl. 1820. 2n = 14
Ecotype sp.: R. macounii Greene
Syn.: *adenosepala Wool. & St., arizonica Rydb., chrysocarpa Rydb., fendleri Crépin, granulifera Rydb., gratissima Greene, grosseserrata*

* *R. aleutensis* Crépin, Bull. Soc. Bot. Belg. **13**:41. 1875. This species has not been mentioned in the North American Flora.

A. Nels., hypoleuca Woot. & St., maximilliana Nees, maximilliana Rydb., mohavensis Parish, neomexicana Ckll., praetincta Ckll., puberulenta Rydb., pyrifera Rydb., salicetorum Rydb., subnuda Lunell (= R. macounii), *terrens Lunell*

Appendix III

Analytical key to the North American species of Cinnamomeae

A. Calyx lobes after flowering usually deciduous. Inflorescence usually glandular.

B. Calyx lobes usually spreading or reflexed in fruit, and falling separately from the disc. Pedicels erect in fruit. Achenes densely hirsute. Habitat, east of 100th meridian west and north to the St. Lawrence basin.

C. Shrubs of bogs and moist shores. Leaflets 7–9, narrow-oblong, acute at each end, finely serrate.

D. Usually 1–2 m. tall, flowering laterals 10 cm. long or more. Inflorescence corymbose.

E. Often armed with flattened infrastipular prickles. No. of teeth on each side of leaf average 26. Stamens over 200. Sepals usually reflexed on the fruit and deciduous. Late flowering

R. palustris

2n = 14

EE. Almost unarmed, prickles weak. No. of teeth average 19. Stamens 150–180. Sepals usually erect and persistent or tardily deciduous. Flowering between *R. blanda* and *R. palustris*

×*R. schuettiana*

2n = 14

DD. Stems low, under 1 m., usually armed with copious reddish bristles. Flowering laterals under 10 cm. long, flowers 1–3 together. Teeth fine, acute, av. no. 16 ..... *R. nitida*

2n = 14

CC. Shrubs of rocky shores, dry uplands, and plains.

F. Stems stout, much branched. Suckers few, rarely

flowering in first season. Leaflets firm and elliptic. Bristles at base and on new shoots, prickles flattened or absent. Teeth 9–30 (av. 14). Stamens 120–155. Newfoundland to eastern Pennsylvania

R. virginiana

2n = 28

FF. Stems usually slender, often bristly to tips, if stout usually simple; often decumbent or bending after first season. Prickles, if present, usually terete. Many suckers which often flower with terminal corymbs after main flowering period. Highly variable and "weedy" species.

G. Leaflets mostly 7, highly varied in shape and texture. Teeth coarse, 5–21, av. 12. Stamens 65–130, av. 105. Hypanthium glandular or smooth

R. carolina

2n = 28

GG. Leaflets mostly 9. Hypanthium often eglandular.

H. Leaflets elliptic to oval, stems bristly to top, erect and simple, or branched and decumbent. Sepals usually spreading in fruit, tardily deciduous or persistent. Stamens 65–143, av. 115 ..... ×*R. rudiuscula*

2n = 28

HH. Leaflets narrow to linear-oblong, acute, glabrous and lustrous above, coarsely serrate. Low and semi-herbaceous, stems almost unarmed. Habitat, southern Great Plains

R. foliolosa

2n = 14

BB. Calyx lobes falling as a group together with the disc. Inflorescence 1–3 flowers. Pedicels drooping in fruit. Stamens 55–72. Achenes few, large, glabrous. Habitat, west of Great Plains ..... *R. gymnocarpa*

2n = 14

AA. Calyx lobes usually persistent after flowering.

I. Twigs, prickles, and bristles finely pubescent.

J. Stems stout, bristly, and prickly, occasionally blooming on the turions. Leaflets large, 2–4 cm., elliptic or narrow-obovate, thick, rugose above. Corymbs small. Fruit usually pendent. Foliage eglandular. . . . . *R. rugosa*
2n = 14

JJ. Plants of Pacific Coast region of U.S.A. Habit varied, 0.3–1.6 m., usually strict. Stems bristly, prickly, and sometimes glandular-hispid. Leaflets small (usually under 2.5 cm.), broadly oval to orbicular, often glandular-muriculate and resin-scented. Inflorescence 1–3. Infrastipular prickles alate, flattened to terete. . . . *R. durandii*
2n = 28

II. Twigs, prickles, and bristles glabrous or merely glandular-hispid.

K. Infrastipular prickles absent.

L. Flowering on laterals from two-year or older wood only. Flowering period short, definite. Upper stipules dilated.

M. Stems unarmed, bristly only at base.

N. Inflorescence 1–2. Petals 1.7–2 cm. Sepals reflexed and persistent on the fruit. Calciphile plants. Bic, Quebec. . . . . *R. williamsii*
2n = 14?

NN. Inflorescence 1–20. Flowers subtended by ovate bracts. Sepals erect or spreading on fruit.

O. Sepals 1.5–2.5 mm. wide, 8–23 mm. long, not narrowed toward base. Leaflets oval to obovate, serrations coarse, acute ascending, av. no. 12. Petals 1–2.5 cm. long. Stamens, av. no. 65. Fruit turning red in 3–4 weeks. Habitat west of 100th meridian. . . . . *R. woodsii*
2n = 14

OO. Sepals 2–4 mm. wide.

P. Small-flowered slender roses found west of Sierra Nevadas. Leaflets ovate with cordate base and fine crenate teeth (av. no. 15). Sepals 2–3 mm. wide, 8–15 mm. long, constricted toward base. Stamens, av. no. 65. Petals 10–18 mm. Fruit needing over 2 months to ripen . . . . . *R. pisocarpa*
2n = 14

PP. Large-flowered eastern roses. Leaflets oval or obovate. Teeth coarse, acute, ascending (av. no. 12). Petals 2–2.8 cm. Stamens 85–140
R. blanda
2n = 14

MM. Stems bristly to tips or nearly so. Leaflets 5–7 (rarely 9). Habitat, Canada and northern U.S.A.

Q. Plants 0.3–2 m. tall. Early flowering.

R. Teeth ovate. Inflorescence 1–3 on laterals less than 7 cm. long. Petals 2.5–3 cm. Stamens 65–125. Early flowering northern plants. Fruits reddening in 3–4 weeks . . . . . *R. acicularis*
2n = 42 and 56

RR. Teeth acute, ascending. Inflorescence 1–20 on laterals usually over 7 cm. long. Stamens 85–140. Flowers in June; fruits reddening in 5 weeks
R. blanda var. *hispida*
2n = 14

QQ. Plants less than 3 dm. high, flowering after hexaploid and diploid species. Bristles short and weak. Flowers usually solitary. Habitat, northern Great Plains . . . . . *R. alcea*
2n = 28

LL. Flowering terminally in corymbs on suckers, as well as laterally on older wood. Everblooming. Flowering period extended.

R. Stems unarmed, usually less than 3 dm. high.

S. Leaflets 5, rarely 7, usually glandular. Hypanthium usually glandular-hispid. Pacific Coast forms ..... *R. spithamaea*
2n = 28?

SS. Leaflets 7–9, coarsely serrate. Flowers solitary or in small corymbs. Flowering on suckers rare. Habitat, northern Great Plains
R. alcea
2n = 28

RR. Stems bristly to tips.

T. Leaflets 5, rarely 7, usually glandular. Hypanthium usually glandular-hispid. Semi-herbaceous dwarf. Habitat, Pacific Coast
R. spithamaea
2n = 28?

TT. Leaflets 7–9 or 11, coarsely serrate. Teeth 7–21. Prairie forms; 0.1–1 m. high. Inflorescence 1–20, terminal or on laterals .... *R. arkansana*
2n = 28

KK. Infrastipular prickles usually present.

U. Stipules at least on the shoots, more or less convolute. Prickles curved. Leaflets pale beneath, eglandular
R. cinnamomea (*introduced*)
2n = 14

UU. Stipules flat.

V. Inflorescence 1–3 on short laterals (3–10 cm. long).

W. Stems bristly at base only, stout with more or less flattened prickles. Foliage often glandular and resin-scented.

X. Stems over 1 m. tall, much branched, with straight ascending or recurved prickles. Petals 2.5–3 cm. Stamens 65–

115. Hip 10–18 mm. in diameter. Fruit firm, needing 10 weeks or more to ripen
R. nutkana
2n = 42

XX. Stems low, 0.2–1 m. Prickles often recurved. Petals 1.5–2.5 cm. Hip 1 cm. in diameter. Southern Rocky Mountain region ..... *R. manca*
2n = 42

WW. Stems bristly to top, slender or, if stout, simple and strict; height to 1 m.

Y. Hips usually erect, pedicels glabrous or glandular, 0.5–2 cm. long. Pacific Coast species.

Z. Stems strict, to 1.6 m., foliage often glandular.

a. Serrations fine, 9–17. Stem and pedicels often glandular-hispid. Prickles straight, flattened or terete. Leaflets usually densely puberulent and resin-scented. Petals 2 cm. long. Stamens about 70
R. durandii
2n = 28

aa. Serrations coarse, 4–14; leaflets 0.5–2.5 cm. Stems with scattered weak bristles and prickles. Petals 12–18 mm.
R. californica var. *bidenticulata*
2n = 28

ZZ. Stems low to 5 dm., with strict branches and numerous weak bristles and prickles. Leaflets to 2 cm. long, oval to orbicular. Petals 1.5–2 cm. Stamens 60–70 ..... *R. yainacensis*
2n = 28

YY. Hips usually pendent. Pedicels slender, 1–3 cm. long.

b. Early flowering. Petals 2.5 cm. Hips 1 cm. or more in diameter, reddening in 5–6 weeks. Stems low, with arching branches. Prickles and bristles slender. Rocky Mountain species

×*R. engelmannii*

2n = 42

bb. Petals 10–15 mm. Hips less than 1 cm. in diameter. Stems slender, branched, prickles very weak. Sepals 4–5 mm. broad. Habitat, mountains of the far west.

c. Sepals rarely caudate, about 10 mm. long, falling together with the disc. Pedicels often glandular-hispid . . . . . *R. gymnocarpa*

2n = 14

cc. Sepals caudate, about 15 mm. long, erect or spreading and persistent on the ripe fruit. Dwarf of southern Sierra Nevadas . . . . . *R. calvaria*

2n = 14

VV. Inflorescence 1–15 or more. Flowering laterals usually over 10 cm.

d. Sepals 1.5–2.5 mm. wide, not narrowed at base. Flowers on laterals from old wood. Buds tapering. Habitat, Great Plains and westward.

e. Leaflets 5–7, 1–4 cm. long, serrations coarse and acute. Twigs with weak, rarely flattened infrastipular prickles. Stems 1–3 m. tall . . . . . *R. woodsii*

2n = 14

ee. Leaflets 7–11, usually under 2.5 cm. long.

Twigs bristly or unarmed. Habit strict. Prairie form, usually less than 1m. tall

R. macounii

2n = 14

dd. Sepals 2–4 mm. wide. Buds ovoid.

f. Small-flowered western roses with arching branches. Leaflets ovate, finely serrate, somewhat cordate at base. Flowers usually in corymbs. Buds ovoid.

g. Stems slender, sparingly armed. Serrations fine and crenate (av. no. 15). Petals 15 mm. long. Sepals narrowed at base. Flowers on laterals only

R. pisocarpa

2n = 14

gg. Stems coarse, often bristly and prickly. Petals 20 mm. long. Sepals slightly narrowed at base. Serrations 10–20 (av. 14). Flowering on long laterals and terminally on turions

R. californica

2n = 28

ff. Large-flowered roses of Great Lakes region. Coarse, strict habit with scattered, flattened prickles. Leaflets oval to obovate. Teeth ascending, 7–28 (av. 17). Petals 2–2.5 cm. Stamens 120–150

×*R. michiganensis*

2n = 14

ORIGIN AND CELLULAR CHARACTER OF XYLEM RAYS IN GYMNOSPERMS

M. W. Bannan

(WITH NINETEEN FIGURES)

Introduction

Although much work has been done on the structure of the xylem ray in gymnosperms, its origin and cellular character apparently have not been investigated from a comprehensive comparative viewpoint. In the present work it is proposed to trace the ray through from its origin, to note the distribution and character of the cells in the different parts of its course, to determine how this distribution and character vary with the location of the ray in the tree (whether in root or stem), and to establish the nature of the changes from the inner to the outer wood. Since *Pinus* affords the greatest variety of cell types, the rays in this form will be described in some detail; the condition in other gymnosperms is considered more briefly.

In the description of rays, the term central, instead of the term interspersed, is applied to the rows in the interior of a ray, the latter term being reserved to describe the alternation in arrangement of different types of cells, in the same radial row, whether that row be marginal or central. Ray cells of the same row, when separated, are referred to as scattered; when in radial contact, as contiguous. Hitherto the term interspersed has also been used to describe the scattered cells of marginal rows.

In the illustrations the pith or primary wood is always toward the left, and the limits of the annual rings are marked by vertical lines. In some of the figures the wall characters are shown; in other figures the ray tracheids and tracheids are represented simply by outlines, "ghosts" by dotted lines, and parenchyma cells by stippling. The stippling merely indicates that the cells are parenchymatous, and does not represent cell content, for in some cases the cells are empty.

Ray origin and cellular character

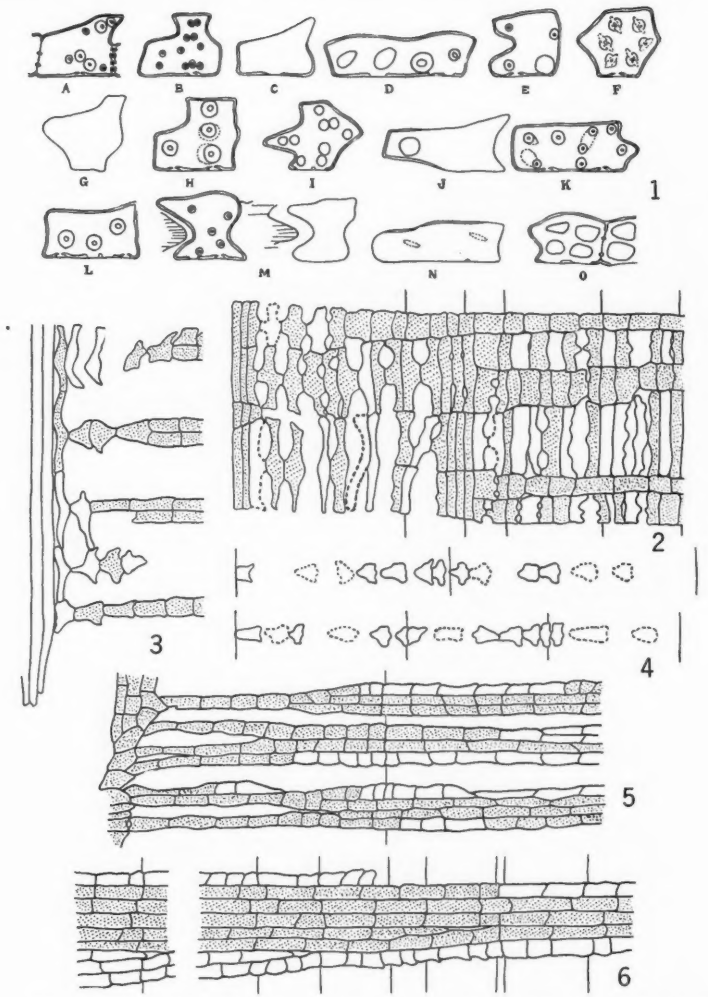
Pinus

In the roots examined, taken from different depths of soil and at different distances from the stem, a considerable amount of variation was observed. It was found that in roots near the stem, or in roots which had been exposed on banks or escarpments, the wood was similar to that in the stem and unlike that in buried roots. Owing to these dissimilarities the two types of roots are considered separately.

In a buried root of a soft pine such as *P. strobus* the rays originating at primary tissue consist of vertically elongated cells, which are either in radial contact or only slightly scattered. Several ray rows are usually found in a vertical series, the earliest cells of each row being in contact with the cells above and below. In some cases the succeeding cells become shorter and the rows separate into individual rays; in other cases there is no shortening and a high ray results. The cells are parenchymatous, although in the succeeding parts an occasional ray tracheid may be interspersed.

Rays arising in the secondary wood are of similar form to those described, but each series of rays succeeds a row of tracheids, with the upper and lower rays of the series in line with the ends of the tracheids, indicating that the ray initials were formed by segmentation of fusiform initials. Although it is usual for the whole fusiform initial to be concerned in the formation of ray initials, occasionally only the upper or lower part is segmented and but one or two ray initials produced. As in the case of the rays beginning at the center, the earliest cells are vertically elongated and are radially contiguous or slightly scattered (fig. 2). Where the succeeding cells become shorter the rows separate into rays, but when such shortening does not occur the rows form a high ray. Most of the rows sooner or later are followed by two or more, the ray initial being horizontally divided.

In the rays originating in the secondary wood, especially in those whose origin is some distance from the center, many different types of cell are found (fig. 1). The earliest cells of the rays are usually tracheary or ghost-like. The ray tracheids as a rule have bordered



FIGS. 1–6.—Figs. 1–4, *Pinus strobus:* Fig. 1, types of ray cell in root wood (dotted lines represent outlines of pores in walls of adjoining tracheids). Fig. 2, portion of ray in root wood. Fig. 3, origin of rays in young stem wood, 0.5 mm. from pith. Fig. 4, new ray in old stem wood 11.5 cm. from pith. The ray is traced through five annual rings. Fig. 5, *P. banksiana,* ray tracheids succeeding parenchyma cells in rays which originated at the pith. Fig. 6, *P. resinosa,* ray in old stem wood 15 cm. from pith showing dying of tracheary rows and development of ray tracheids in parenchymatous row.

pits in all walls touching other cells, but there is considerable variation in the size of the pits, as indicated in types *B*, *H*, and *L*. Other cells may have both tracheary and parenchymatous characters, such as bordered and simple pitting, with the simple pits on radial walls (*D*, *E*), horizontal walls (*D*, *L*), or tangential walls (*A*). There may also be irregularities in the pits of the ray cells and the adjoining tracheids, both in size and character of the pits. For instance, simple or but slightly bordered pits in the tracheids may overlie bordered pits in the ray cells (*H*, *K*). Interspersed among the tracheary cells are many ghost-like types. These show gradations from faint outlines (*G*) on the one extreme to parenchyma cells with small pits (*I*) opposite bordered pits in the neighboring tracheids, on the other extreme to parenchyma cells with large pits (*O*) opposite simple pits in the tracheids. In the intermediate types the walls are thickened and may be unpitted (*C*, *N*) or pitted (*J*). The ghost-like cells, ray tracheids, and many of the parenchyma cells lose their protoplasm shortly after differentiation. The wall character of the ordinary parenchyma cell is shown in (*O*).

In addition to the ghosts, intercellular tracings or markings (fig. 1, *M*) often occur near the origins of rays, where the cells are vertically rather than radially extended. These are probably indicative of a displacement of ray cells in relation to tracheids, resulting from different rates of division of ray and fusiform initials and a failure of the ray cells to extend radially.

The rays beginning at the center of the root are usually entirely parenchymatous throughout their course, although in a few cases ray tracheids may be interspersed among the parenchyma cells in that part of the ray some distance from the center. On the other hand, the rays originating in the outer secondary wood are at first composed of tracheids and ghost-like cells with parenchymatous types interspersed. In the succeeding parts of these rays the parenchyma cells become more numerous and eventually the rays are composed wholly of this type of cell. Outward from the center there is an increasing development of ray tracheids, principally in the first formed parts of the successive new rays, but also to a lesser degree in the parenchymatous rays of early origin.

In exposed roots the rays resemble those in the stem, and as the

latter will be described in some detail, the rays in this type of root will not be further considered. Between stemlike roots on the one hand and buried roots on the other there are many intermediate gradations.

Rays arising at the pith of the stem of *P. strobus* consist of contiguous parenchyma cells. As in the root, several ray rows are usually found in a vertical series, the earliest cells of each row being vertically elongated and in contact with the cells above and below. The succeeding cells shorten more rapidly than in the root, however, and the rows soon separate into rays.

The rays originating in the innermost secondary wood are of like character, but at a distance of 0.3 mm. or more from the pith a change is noted. Ray tracheids appear in some new rays and occasionally also in rays which began at the pith. When in new rays they may be either the first cells or be preceded by one or two ray parenchyma cells (fig. 3), but in any case there are few ray tracheids in a single ray. A slight scattering of the first cells of the rays may also be observed.

In the outer, as in the inner wood, the new rays as a rule are found in a vertical series; but here, in contrast to the condition in the inner wood, there are considerable gaps between the last tracheids and the first cells of the succeeding new rays. The earliest ray cells are scattered and there is little or no transition from vertically to radially elongated elements, even the first cells being short and somewhat radially extended (fig. 4). The new rays are usually only a single cell high, and the cells are for the most part tracheary, although ghosts may be interspersed. In the succeeding parts of the ray the cells are longer, and also closer, so that they come into contact. The initial of the ray, which may yet be a single cell high, is then horizontally divided, sometimes successively, and the number of rows increased. While the ray is increasing in height the tracheary character continues in the marginal rows, and the central rows on the other hand become parenchymatous. In the outer wood from 30 to 50 per cent of the total ray tissue is tracheary.

In the stem the newly formed ray initials shorten more rapidly than in the root. The ray initials formed by the segmentation of a single fusiform initial soon separate, producing a number of separate

rays one cell in height rather than one or two rays several cells high, such as often occurs in the root. The older rays in both root and stem add to their height by doubling some of the rows, but this doubling occurs more often in the root. Consequently, owing to the greater initial height and the faster rate of increase, the rays in the root are much higher than in the stem.

Outward from the center in both root and stem there is an increasing development of ray tracheids, mostly in the earliest parts of the new rays, but also in the succeeding parts of the formerly parenchymatous rays which originated at the center. There are, however, considerable differences in the numbers of the various types of cell between root and stem. In the stem there are many more typical ray tracheids, and in proportion to these, fewer poorly developed ray tracheids, fewer ghost-like cells, and fewer cells intermediate in character between ray tracheids and parenchyma than in the root.

Compared with the soft pines, in the hard species ray tracheids appear earlier, are more abundant in old wood, and are of more elaborate form. Unlike the soft pines, ghost-like cells are scarce in the root, and are rarely found in the stem. There are likewise fewer cells with bordered and simple pitting, etc. Practically all the cells are either typically tracheary or parenchymatous, the former constituting 50 per cent or more of the ray tissue in old stem wood.

The sequence of the two principal types of ray cell, tracheary and parenchymatous, has been a subject of controversy among anatomists. In the pines KNY (**6**) illustrated the formation of ray tracheids, either as new rows or as succeeding parenchyma in older rows. SCHMIDT (**9**) and THOMPSON (**10**) described the origin of new tracheary rays, THOMPSON stating that ray tracheids were ultimately followed by parenchyma. PENHALLOW'S theory (**7**) on the origin of ray tracheids from parenchymatous tissue was interpreted by THOMPSON as necessitating an actual sequence of parenchymatous cells followed by ray tracheids, but he did not find such an arrangement.

As noted in the foregoing description, the rays originating in the secondary wood are at first tracheary, or predominantly tracheary (parenchyma cells may sometimes be interspersed among the ray tracheids), and later become parenchymatous. The reverse order,

that of parenchyma followed by ray tracheids, occurs in addition however. In young stem wood many of the marginal parenchymatous rows of rays originating near or at primary tissue are succeeded by tracheary rows (fig. 5), this sometimes occurring to such an extent in the hard pines that even close to the pith half the ray rows become tracheary. Many of the tracheary rows which succeed parenchyma are in turn followed by parenchyma in old wood.

The sequence of parenchymatous followed by tracheary cells may also be seen in old stem wood. Such change frequently occurs in submarginal rows of old rays, in which case it is usually correlated with the cessation of the adjoining marginal row (fig. 6). The upper marginal tracheary row has ceased, and the submarginal parenchymatous row has become tracheary after assuming a marginal position. In some cases a row such as the latter in turn dies and the parenchymatous row within becomes a marginal tracheary one. Sometimes the submarginal row attains tracheary characters before the marginal row ceases, and instances were observed where the change took place in the submarginal row although the marginal one continued unaltered. The sequence from parenchymatous to tracheary cells occurs less frequently in the central rows. As in the marginal rows, the change is sometimes associated with the dying of the row; that is, when a parenchymatous row ceases some of the last cells may be tracheary instead of parenchymatous. In other cases a few ray tracheids are simply interspersed in otherwise normal parenchymatous rows.

Although the dying of tracheary ray rows is of common occurrence in old wood, it occurs more often in the slowly growing parts. This relation was shown most clearly in an old tree of *Pinus resinosa* which was growing in a burnt-over area and had a serious fire wound at the base of the stem. The growth rings preceding the year of the fire were wide but afterwards were very narrow. In a given area in the growth ring preceding that of the wounded year, but some distance above the wound, there was a net gain of ten ray rows whereas in the corresponding area in the five narrow years there was a net loss of forty-two rows. In the six years fifty-five rows ceased, of which fifty-four were tracheary. Owing to the fact that some parenchymatous rows became tracheary, however, the percentage reduc-

tion of tracheary tissue was relatively slight, from 51 to 46 per cent. Similar relations between the ring width and the character of the ray tissue were noted in slowly growing but unwounded trees.

The last ray tracheids in the dying rows are of various shapes. Sometimes they are long and radially attenuated; in other cases they are shorter than usual. The latter condition is illustrated in the upper row in fig. 6. After the last ray tracheid there are as a rule no ghosts. Exceptions to this condition are rare, and are for the most part limited to such locations as the dying rows of recently formed rays in soft pines, where ghosts occur in any case. The lack of ghosts in dying tracheary rows is of special significance in the interpretation of ghost-like cells.

Other gymnosperms

In the case of the other gymnosperms studied, the rays in the inner stem wood are composed of contiguous parenchyma cells, with the earliest cells of each ray showing gradations from vertically to radially elongated elements, such as described in *Pinus*. From the rays of this character, found in the inner wood, there is a sequence to that type of ray peculiar to the species in old wood.

In the roots the transitional stages from the first high cells of the new rays to the succeeding radially extended ones last longer than in the stem. The rays are higher, and even in old roots are more largely parenchymatous than in stems of like size or age. Sometimes the differences in cellular character of the rays in root and stem are very great. For instance, in *Picea*, where ray tracheids constitute 30 per cent of the ray tissue in mature stem wood, old roots of the buried type were found which were entirely lacking in ray tracheids, and in others these cells occurred only sporadically.

Since the rays in the innermost stem wood and in the root of these gymnosperms are of more or less uniform origin and cellular character they will not be further considered. There is, however, much variation in the shape, arrangement, and character of the ray cells in old stem wood. Consequently the condition in the old stem will be more fully dealt with, the forms with parenchymatous rays being described first.

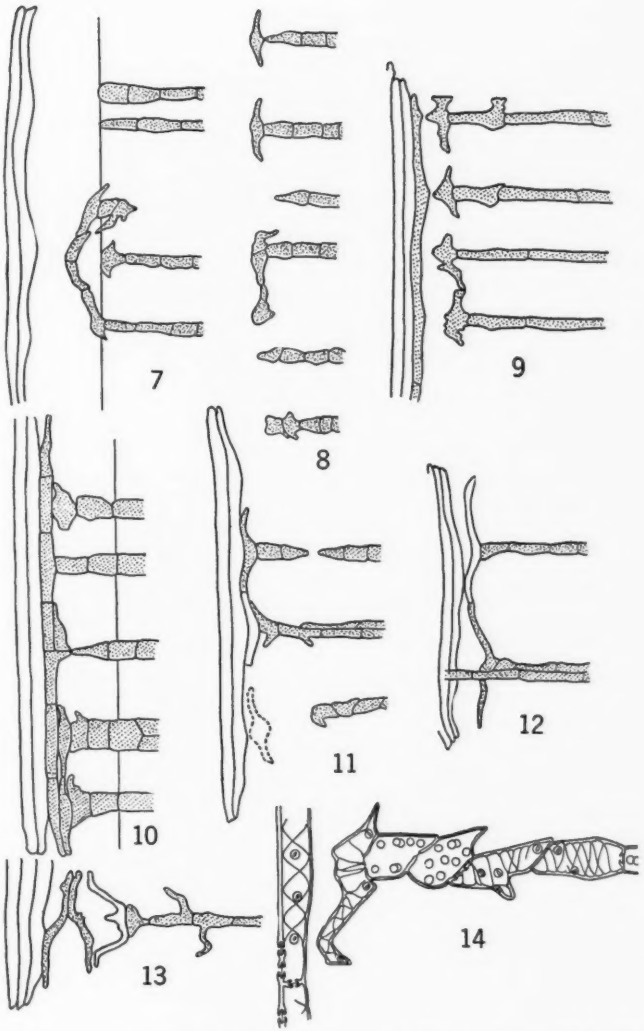
In the Cycadales *Dioon spinulosum* was selected, because of the

width of the zone of wood. The new rays succeed radial rows of tracheids or xylem parenchyma, with the first cells of the rays in contact with or close to the last fusiform elements. All the ray cells are contiguous and parenchymatous.

The new rays in old wood of *Ginkgo biloba* succeed tracheids, septate tracheids, or xylem parenchyma, with or without short gaps between the rays and the fusiform cells (fig. 7). The first ray cells are sometimes vertically extended, but more often are short and somewhat elongated radially, the transitions from vertically to radially elongated cells being largely omitted. The earliest cells of the rays are usually parenchymatous and thin-walled, but in rare instances are thick-walled, and in very exceptional cases may be tracheary. The succeeding cells are contiguous and always parenchymatous.

Rays in stem wood of the Araucarineae (fig. 8) are similar to those in the stem of *Ginkgo*, except that the gap between the last tracheids and the first ray cells may be slightly greater, the earliest cells are more often radially extended, and typical ray tracheids occur though only sporadically. The rays are usually composed entirely of thin-walled parenchyma, but in rare instances a few of the earliest cells are tracheary. These cells were found only near the ray origins, a distribution which is different from that noted by POOL (**8**), who described them in the central rows of old rays. Ghosts may be observed in an occasional new ray but their numbers are exceedingly small. Thick-walled parenchyma cells are sometimes present, both in new and old rays.

In the Podocarpineae, *Dacrydium*, *Microcachrys*, *Phyllocladus*, *Saxegothaea*, and *Podocarpus* were studied. The origin of rays in the stem is illustrated in figure 9. The new rays succeed tracheids or xylem parenchyma, with little or no gap between, and consist of contiguous parenchyma cells, the earliest of which show transitions from vertically to radially elongated shape. The only species in which ray tracheids were found was *Microcachrys tetragona*. Here they were of rare occurrence, having been found only in new rays, with never more than one tracheid in a single ray. The tracheary containing rays were sparsely distributed throughout the wood of the stem and showed no correlation with injury. The ray parenchyma cells in the Podocarpineae have different degrees of wall thickening



FIGS. 7–14.—Newly formed rays in stem wood: Fig. 7, *Ginkgo biloba*. Fig. 8, *Agathis alba*. Fig. 9, *Podocarpus elatus*. Fig. 10, *Torreya nucifera*. Fig. 11, *Taxus baccata*. Fig. 12, *T. brevifolia*. Fig. 13, *T. baccata*. Fig. 14, *T. canadensis*, showing tracheary cells in ray.

and lignification, and there is considerable variety in the pitting between ray cells and tracheids.

The rays of such Taxineae as *Cephalotaxus* and *Torreya* exhibit features similar to those of the Podocarpineae. The earliest cells of the rays are usually vertically elongated and the following ones lower (fig. 10). The ray cells are almost invariably contiguous and are parenchymatous.

In *Taxus* the rays succeed tracheids with little or no gap intervening. The first cell of the ray is frequently very high, and the following ones much shorter, the transition from high to low cells being quickly passed over. In the majority of the rays all the cells are parenchymatous, but in some cases one of the earlier cells is tracheary, as in figures 12 and 13, and in a few cases typical ray tracheids are present (fig. 14). The ray tracheids are always near the ray origins but are not necessarily the first ray cells, there being no fixed sequence of ray tracheids and parenchyma. Not more than three ray tracheids were found in a single ray, and the rays with such cells were few in number, occurring sporadically throughout the stem. The walls of the tracheary ray cells show spiral thickenings and as a rule have few pits, the tangential walls sometimes lacking them entirely. Ghosts, of which one is shown in the lower ray in figure 11, are of rare occurrence.

It has been mentioned that the earliest cells of most of the new rays are in contact with or only slightly removed from the preceding fusiform elements, and that the ray cells themselves are contiguous. This arrangement is significant for it indicates that there can be no question of ray tracheids having been once present but lost by dropping out.

In the different Cupressineae studied a considerable amount of variation was found in the rays of old stem wood. Here, as in old wood generally, the new rays are usually one cell high. In some cases the majority of the cells of the new rays are parenchymatous, with a few ray tracheids interspersed, and in others the rays are largely tracheary, with parenchyma interspersed. The earliest cells are radially extended and scattered, with gaps between them and the last of the preceding tracheids. The gap is usually widest and the scattering greatest in the rays where the tracheids are the most nu-

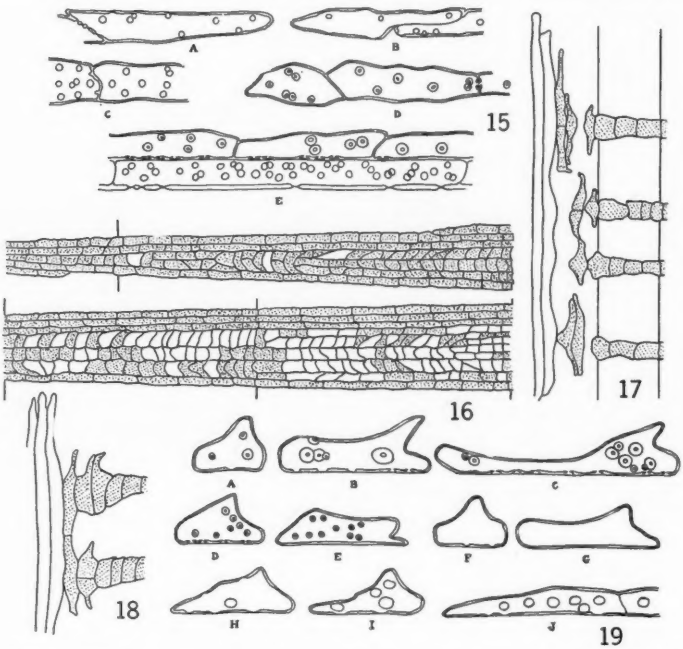
merous. When the ray is traced outward in the older wood the parenchyma cells interspersed among the tracheids increase until the ray becomes entirely parenchymatous, and at this point the cells are usually contiguous. The initial of the ray, which as a rule is still a single cell high, may then be horizontally divided, producing a parenchymatous ray two cells or more in height. Where a marginal row of an old ray is tracheary this is due to the addition of an entirely new ray to the margin, rather than to a continuance of the tracheary character in the ray itself.

Ray tracheids were abundant in the material of some of the species studied and rare in others; but since similar variation was observed in those forms where a choice of material was available, such as *Thuja* and *Juniperus*, it is evident that the differences cannot be considered necessarily specific or even generic. Ray tracheids were found in varying number in stem wood of all the genera studied (*Callitris*, *Chamaecyparis*, *Cupressus*, *Juniperus*, *Libocedrus*, *Thuja*, *Widdringtonia*), and the greatest number noted was in an old stem of *Thuja occidentalis* where 6 per cent of the ray rows contained tracheary cells.

Some of the different types of cells found in new rays are illustrated in figure 15 from *Thuja occidentalis*. The ray tracheids have large, or small, or both large and small bordered pits on radial walls but frequently lack them on tangential walls (*D*, *E*). The majority of the parenchyma cells have thin tangential walls (*B*, *E*) but a few have thickened ones (*A*, *C*). Their radial walls are usually abundantly pitted (*C*, *E*) but are occasionally only sparingly so (*A*, *B*). Some of the parenchyma cells, particularly those with few pits such as the *A* and *B* types, are characterized by the early disappearance of their protoplasm. Empty thin-walled unpitted cells and ghosts were not observed. The older rays are composed of ordinary parenchyma cells with thin tangential walls, abundantly pitted radial walls, and intact protoplasm (*E*).

In addition to the rays described, others of a peculiar type were occasionally found in *Thuja occidentalis*. One of these is illustrated in figure 16. The abnormal feature is the development of ray tracheids in the central rows of the ray, which in this particular case is all the more outstanding since the ray originated at primary tissue. Where

ray tracheids succeed parenchyma in the pines, it is usually either in marginal rows or in dying central rows; but neither of these conditions holds in *Thuja*. The abnormal rays were found in both young and old stem wood, but considering the large number of specimens examined are rather rare. No correlation with wounding was evi-



FIGS. 15–19. Figs. 15–17, *Thuja occidentalis:* Fig. 15, types of ray cell in old stem wood. Fig. 16, abnormal ray with ray tracheids in central rows. Fig. 17, newly formed rays in root wood, illustrating character of rays in root and young stem wood. Fig. 18, *Cunninghamia lanceolata*, character of new rays in young stem wood. Fig. 19, *Sequoia sempervirens*, types of ray cell in old stem wood.

dent. Rays of like character were not observed in other species, although HOLDEN (**4**) has described somewhat similar rays from *Sequoia gigantea* and POOL (**8**) from *Araucaria*.

In the Taxodineae the differences in ray structure are apparently recognizable as generic. Based upon the material examined, which in some cases was rather limited, the genera are divisible into three

groups: *Athrotaxis* comprises one, *Taxodium* and *Cryptomeria* the second, and *Sequoia*, *Cunninghamia*, and *Taiwania* the third.

New rays throughout stem wood of *Athrotaxis* almost invariably consist of contiguous or only slightly scattered parenchyma cells, with the earliest cells close to or in contact with the preceding tracheids. Ghosts and thin-walled sparsely pitted empty cells are apparently absent, and ray tracheids very rare.

In *Taxodium* and *Cryptomeria* the new rays consist of scattered cells which are either ghost-like or have thin, sparsely pitted walls and are devoid of content. Ray tracheids occur only rarely, interspersed among the ghosts or empty cells. In the succeeding parts of the rays, thicker walled empty cells, resembling types *F* to *J* in figure 19, appear, and so increase as to constitute the bulk of the tissue. These cells in turn are followed by contiguous parenchyma cells of ordinary character. The ghosts, empty cells, and occasional ray tracheids comprise from 5 to 10 per cent of the ray tissue in old wood.

Sequoia, *Cunninghamia*, and *Taiwania* differ from *Taxodium* in having fewer ghosts and more abundant ray tracheids. If a ray arising in old wood is followed through from its origin, it is found that the earliest cells are scattered and mostly tracheary; but outward there is an increase in interspersed parenchyma and cells combining tracheary and parenchymatous characters, accompanied by diminishing spaces between the cells, until the ray is entirely made up of contiguous parenchyma. The old rays are several cells high and are usually wholly parenchymatous, although sometimes ray tracheids may be observed in the marginal rows. In most cases, however, the latter condition is due, not to a continuation of the tracheary character through from the origin of the ray itself, as in the pines, but to the addition of entirely new rays (which are tracheary like all new rays in old wood) to the margin of the older ray.

The different types of ray cells in these forms are illustrated in figure 19 from *Sequoia sempervirens*. The majority of the ray tracheids have bordered pits in all walls touching other cells (*C*), but in some cases lack them on tangential or horizontal walls (*E*). The pits vary considerably in size (*B*, *C*). Other cells, such as described by GORDON (3), have both bordered and simple pits (*A*, *B*), with the

simple pits on horizontal walls adjacent to ray parenchyma. Ghosts are rare, and cells with slightly thickened and lignified but unpitted walls are of occasional occurrence. From the latter one may observe gradations in cells with thickened but sparsely pitted walls (*F*, *H*, *I*), the majority of these being without content. Although all these types may be recognized in new rays, most of the cells are either typical ray tracheids or parenchyma. Approximately 10 per cent of the ray rows in old wood of *Sequoia sempervirens*, and 5 per cent in *S. gigantea*, *Cunninghamia*, and *Taiwania* are largely composed of tracheary cells, the remainder of the rows being wholly parenchymatous.

Old wood of *Sciadopitys* was not available, but in young stem and root wood the rays were entirely parenchymatous, as in the similar regions of the other Taxodineae (fig. 18).

The cells in the newly formed rays in old wood of some Taxodineae are widely scattered, more so than in other conifers. Where such scattering is found, outlines of cells never occur in sufficient numbers to make the rows contiguous. Occasionally the separated ray cells have ghost-like pithward-extending appendages, which obviously are not vestiges of separate cells but are evidence that the ray cells and tracheids have moved in relation to each other. Sometimes the tracheid lying side by side with and just preceding a ray cell in the scattered row has small pits on that part of its wall in line with the ray cell Such phenomena indicate that the extent of the scattering is dependent upon the rate of division of the ray initials, rather than upon the breakdown and disappearance of cells. The spaces cannot be interpreted as the result of a loss of ray tracheids, which cells some investigators consider have largely disappeared from the Taxodineae.

The distribution of ray tracheids in the Taxodineae and Cupressineae has been dealt with by several investigators, but most extensively by HOLDEN (4). She has described ray tracheids in several species as being more abundant in wounded material, and considered these cells traumatic. JEFFREY (5) likewise believes ray tracheids to be traumatic reversions to an ancestral condition. The writer, however, was unable to find evidence in support of such interpretation. Wounded specimens of several genera (*Callitris*, *Cupressus*, *Chamae-*

cyparis, *Juniperus*, *Thuja*, *Sequoia*, *Taxodium*, and *Cunninghamia*) were examined, but an increase in ray tracheids, either in the parts adjacent to or opposite the wounds, was not found when comparison was made with normal wood.

In the sub-tribe Abieteae of the Abietineae generic differences in ray structure are further accentuated. Rays in mature stem wood of *Keteleeria* contain numerous ghosts but no ray tracheids. *Abies* is similar except that ray tracheids are present, although rare. *Cedrus* has fewer ghosts and more ray tracheids, and in *Tsuga* there are exceedingly few ghosts and abundant ray tracheids.

The cells of the newly formed rays in *Keteleeria* are scattered and of the following types: ghosts, empty cells with slightly thickened but unpitted walls, gradations from these to parenchyma with typical wall structure but devoid of content, and finally ordinary parenchyma. The ghosts are most numerous toward the ray origin, and the other types become more abundant in the succeeding parts. In old rays the ghost-like character may continue in the marginal rows, the ghosts occurring in the spring and parenchyma cells in the summer wood of each annual ring, while the central rows are exclusively parenchymatous. From 20 to 25 per cent of the ray rows in old wood contain ghosts, the remainder being parenchymatous.

In the case of *Pseudolarix* old stem wood was not studied, but in young material the rays resembled those in *Keteleeria* of like age.

The rays of *Abies* are similar to those of *Keteleeria*, except that the changes in ray character outward from the pith are of more rapid sequence and that ray tracheids are of sporadic occurrence. Cells with both bordered and simple pits, as described by THOMPSON (11) from *Abies homolepis*, occur rarely. The ghosts, thin-walled empty cells, ray tracheids, etc., constitute approximately 25 per cent of the ray tissue in old wood.

The erratic nature of the distribution of ray tracheids in *Abies* is evident from the work of several investigators. PENHALLOW (7), DEBARY (2), and WIESEHUEGEL (12) noted ray tracheids in *A. balsamea*, but HOLDEN (4) and THOMPSON (11) failed to find any in this species. THOMPSON described these cells in *A. amabilis*, *A. concolor*, *A. homolepis*, and *A. veitchii*, however, and considered them traumatic. Extensive material of *A. balsamea* was examined by the

writer and ray tracheids were found sparingly in a few specimens but were absent from the majority. Although sometimes occurring in wounded areas, the ray tracheids were absent from many other wounded specimens, and present in others which showed no evidence of injury. The distribution could not be correlated with wounding or vigor of growth. Ray tracheids were observed also in *A. concolor*, *A. homolepis*, *A. lasiocarpa*, and *A. veitchii*. Here again they were not necessarily connected with injury. In fact, in a wounded specimen of *A. concolor*, a species in which THOMPSON described traumatic ray tracheids, these cells were actually less numerous in the wounded year's growth than in the preceding unwounded wood.

The cells in the rays of *Cedrus* are of similar types to those in *Abies*, but ghosts are less numerous, the majority of the cells in the first formed parts of the ray being tracheary. The ray tracheids, ghosts, thin-walled empty cells, and interspersed parenchyma of various types comprise from 12 to 25 per cent of the ray tissue in old wood.

In contrast to other Abieteae, ghosts are rare in *Tsuga canadensis*. The earlier cells of the rays are usually somewhat scattered and are tracheary almost without exception. With subsequent increase in height of the ray, the tracheary character persists in the marginal rows; although in this part of the ray parenchyma cells are often interspersed among the ray tracheids, especially in summer wood. Approximately 30 per cent of the ray rows in old wood are tracheary.

Tsuga caroliniana resembles *T. canadensis*, but in *T. heterophylla* and *T. mertensiana* the first ray tracheids appear farther outward from the pith; and in old wood these cells constitute 15 to 25 per cent of the ray tissue, about 10 per cent less than in *T. canadensis*.

In the sub-tribe Pineae, *Larix*, *Picea*, and *Pseudotsuga* have similar rays. As in *Tsuga*, the cells of the new rays are somewhat scattered, with the first cells some distance from the preceding tracheids. The new rays are a single cell high and are exclusively tracheary. In the succeeding parts, where the ray is higher, the tracheary character present in the earlier part of the ray continues in the marginal rows, and the central rows become parenchymatous. In some cases, as noted in the Cupressineae and Taxodineae, new tracheary rays may be added to the margins of old rays, but in *Picea*, *Larix*, *Pseudotsuga*, and also in *Tsuga* the majority of the tracheary marginal rows may

be traced back to the origin of the ray itself. Unlike the condition in *Tsuga*, parenchyma cells are rarely interspersed among the ray tracheids, unless as vertical resin canals. Practically all the cells are either typical ray tracheids or parenchyma; ghosts, thin-walled empty cells, etc., are rarely found. Usually 30 per cent, but at times as much as 50 per cent of the ray rows are tracheary.

In the rays arising in the old wood, the earliest parts are tracheary and the succeeding parts (in the central rows) parenchymatous. The sequence of cell types for the central rows is that of ray tracheids followed by parenchyma. The reversed order, that of parenchyma cells succeeded by ray tracheids, may also be observed in *Larix*, *Pseudotsuga*, *Picea*, and to a lesser degree in *Tsuga*, under such circumstances as have been described in the pines. In all the other conifers ray tracheids occur almost invariably only in new rays and, except for the parenchyma cells interspersed among ray tracheids, very rarely succeed parenchyma.

Discussion

In the description of rays evidence has been given for the formation of ray initials by segmentation of fusiform initials. In the behavior of the ray initials, however, and also in the character of the daughter ray cells, there is considerable variation. The differences will be further considered and their significance discussed.

In stem wood of a gymnosperm such as *Dioon*, the ray initials formed by the segmentation of a single fusiform initial lie in a vertical series, each of the initials touching those above and below. The ray initials continue dividing at approximately the same rate as the neighboring fusiform initials, and in consequence the earliest cells of the rays are high and narrow and in contact with similar cells above and below. Subsequently the initials shorten and separate. They divide more slowly, and in the succeeding parts of the rays, which have now separated, there are graded series from the first vertically elongated cells to those of lower and somewhat radially extended shape. All the cells produced by the ray initials are parenchymatous. This type of ray is found in *Dioon* and, with slight modification throughout stem and root wood of the Taxineae and Podocarpineae, and in the root and innermost stem wood of most conifers.

The second type of ray is characterized by the appearance of gaps

between the last fusiform elements and the first ray cells, and by the omission of some or all of the transitions from vertically to radially elongated cells. In some cases the earliest cells of the rays are radially separated, this being the result of slow ray initial division. All the ray cells are parenchymatous. Such rays are found in mature stem wood of *Ginkgo*, the Araucarineae, and some of the Cupressineae and Taxodineae.

In the third type the general form of the ray is similar to that just described, but some of the earlier cells are tracheary instead of parenchymatous. Rays of this type occur sporadically throughout mature stem wood of certain Araucarineae, Podocarpineae, and Taxineae, in greater numbers in Cupressineae and Taxodineae, and most abundantly in young stem wood of the majority of the Abietineae.

The changes in the character of the ray origin, noted in the second and third types of rays, are accentuated in the fourth type. The gaps between the first ray cells and the preceding tracheids are often of considerable extent, and the transitional stages from high to low and radially extended cells are usually entirely omitted. The majority of the earlier cells of the rays are tracheary. With the increased development of ray tracheids these cells, which at first are found only in the earliest parts of the rays, extend into the succeeding parts and, at the expense of the parenchyma cells, constitute the marginal rows of the older rays. Rays of this character may occur sporadically in old stem wood of such Taxodineae as *Sequoia*, and are characteristic of the mature wood of most of the Abietineae.

Ultimately ray tracheids appear also in ray rows which were formerly parenchymatous, both in rays originating in the secondary wood and in rays arising at the pith. Such development is best seen in the stem of the hard pines, but also occurs in *Picea*, *Larix*, *Pseudotsuga*, and to a limited extent in *Tsuga*. The appearance of ray tracheids in parenchymatous rows beginning at the pith is of particular significance, for it indicates that the tracheary character may be attained by all ray cells regardless of the point of ray origin.

There is then a sequence from parenchymatous rays, composed of contiguous cells, in such admittedly primitive gymnosperms as the cycads, to tracheary rays with scattered cells (lacking the transitions from vertically to radially elongated cells) in such Abietineae

as the pines. This sequence in its general features parallels that outward from the inner to the outer wood in pines and other conifers with ray tracheids in mature wood. Further, in the fossil forms, except those of Abietinean affinity, there is a general absence of ray tracheids. These parallels in sequence from parenchymatous to tracheary rays therefore indicate that ray tracheids are a newer type of cell appearing in the ray in place of parenchyma.

The view that ray tracheids replace parenchyma, and that a sparing occurrence of the former is indicative of initial stages of acquirement, was advanced by PENHALLOW (7). On the other hand JEFFREY (5), HOLDEN (4), THOMPSON (11), and CHRYSLER (1) have presented evidence which they consider indicative of a loss of ray tracheids. Their conclusions were based upon such features as the occurrence of ray tracheids in or near wounded areas, and on the interpretation of ghosts and parenchyma cells interspersed among ray tracheids. The bases of such interpretation will be more fully considered.

The writer examined wounded specimens of several genera but an increase in the number of ray tracheids was not observed when comparison was made with normal wood. The locations in which traumatic ray tracheids have been described, such as the wound cap and the side opposite the wound, were studied in particular, but ray tracheids were not found to be more abundant there than in other parts of the tree. Evidently ray tracheids are to be considered characteristic of normal wood, rather than traumatic reversions.

In regard to the ghosts described by THOMPSON in *Abies* and considered by him to represent vestigial ray tracheids it is significant that these are found abundantly in not more than six or seven genera of the conifers, and if such a loss of ray tracheids has taken place it has been only in these few genera, belonging to the Abieteae and Taxodineae. In all the other gymnosperms the rays are composed either of parenchyma cells, or of parenchyma and ray tracheids and their various intergrades, typical ghosts being scarce or absent. Hence if these forms (Araucarineae, Podocarpineae, Taxineae, Cupressineae, and most Taxodineae) have lost ray tracheids, such cells must have been replaced by parenchyma. On the contrary, the evidence of phylogeny and of the ontogenetic sequence indicates that the replacement has been one of parenchyma by tracheary cells.

From a theoretical standpoint it is also of interest to note that in such regions as the female cone axis and the root, which have been regarded by a number of investigators as conservative, ray tracheids are absent or less numerous than in the stem.

Summary

1. The origin and cellular character of rays originating at different points in the xylem of stem and root were studied from representative gymnosperms.

2. The ray initials evidently are formed by segmentation of fusiform initials.

3. The newly formed rays in *Dioon* are wholly parenchymatous, as are also the majority in *Ginkgo*, Araucarineae, Podocarpineae, and Taxineae. In the stem of the latter groups ray tracheids occur sporadically in occasional new rays.

4. Rays in the inner wood of the Cupressineae, Taxodineae, and Abietineae are parenchymatous, but in old stem wood ray tracheids occur in varying degrees of abundance. In the Cupressineae and Taxodineae the ray tracheids as a rule are found only in new rays, but in the Abietineae they appear also in rays which originated earlier and which were formerly parenchymatous.

5. Roots are variable in structure. In buried roots the rays are more largely parenchymatous and less tracheary than in the stem, while in exposed roots the rays resemble those in the stem.

6. The sequence in ray origin and cellular character outward from the pith in living forms, the condition in the different groups of gymnosperms, and the fossil evidence, all indicate that the primitive ray is parenchymatous and that ray tracheids have arisen at the expense of parenchyma.

I wish to express gratitude to Professor R. B. THOMSON, both for his kindly criticism and assistance and also for the material made available by him. For supplementary material I am indebted to Professor J. H. WHITE of the Faculty of Forestry.

UNIVERSITY OF TORONTO
TORONTO, CANADA

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STUDIES ON THE GERMINATION OF THE SPORES OF CERTAIN BASIDIOMYCETAE

FRED H. O. KAUFMANN

(WITH TWO FIGURES)

Introduction

This investigation was undertaken for the purpose of determining the influence of various nutritional relations, of hydrogen-ion concentration, of temperature, and of other factors upon the germination of the spores of certain species of three families of the Basidiomycetae, the Agaricaceae, Nidulariaceae, and Lycoperdaceae.

The earliest attempts at germinating the spores of fungi are generally credited to PREVOST in 1807, EHRENBERG in 1821, CORDA in 1842, and to others. HOFFMANN'S work (**14**), however, published in 1860, was the earliest extensive investigation of this character and seems to mark the initiation of the use of the hanging drop technique, which, improved upon by others, has gradually developed into a standardized procedure centering around the Van Tieghem cell. HOFFMANN'S results were so definite that he was able to demonstrate the effect of environmental factors and of internal factors, such as age, upon the germination of the spores; but he was unable to induce the germination of the spores of the Lycoperdaceae. BREFELD (**3**) and DE BARY (**8**) found the same negative response a few years later.

Decoctions of manure, fruits, and other materials were the favored media of those studying the germination of spores during this early period, the use of media of known composition coming later. DUGGAR (**9**) used a variety of media, and observed that glycerin as a source of carbon yields higher germination percentages of the spores of certain saprophytic fungi than does sugar, and that neutral salts give greater stimulation than do acid ones.

FERGUSON (**11**) studied the effect of types of nutrition, reaction of medium, temperature, light, and age upon the germination of the spores of *Agaricus campestris* and many other species of saprophytic Basidiomycetes. She succeeded in securing germination percentages

as high as 85 per cent, and demonstrated that temperature extremes constitute the only special stimulus required to induce germination in some instances. FERGUSON also attempted to induce germination of puffball spores, but without success. SWARTZ (18) reports that COOL also failed to germinate the spores of several species of puffballs. SWARTZ himself claims to have germinated the spores of *Calvatia saccata* and of *Lycoperdon pyriforme* after exposing them to alternate wetting and drying.

From about 1904, the majority of workers have attacked the problem of germination of spores of certain refractory saprophytic Basidiomycetae by determining the influence of the digestive system of animals upon their capacity for germination (13, 1, 4, 5). STYER (17) and BECHMANN (2) confined their investigations chiefly to studying the influence of nutritional and other physiological factors upon the germination of the spores of the commercially important mushroom, *Agaricus campestris*.

Of special interest in this connection is the early recognition of the importance of the reaction of the medium to the germination of fungous spores, culminating in the quantitative studies of WEBB (19) and others. FINDLAY (12), following up the earlier work of FALCK, secured positive results with *Merulius lachrymans* only in acid media, while EVANS and HARRAR (10) showed the influence of pH in the germination of the oospores of *Sclerospora graminicola*.

Technique

SPORES.—The spores used in this work were aseptically removed from the fruiting bodies of the following species of Basidiomycetae collected in and about the city of Madison, Wisconsin: *Coprinus micaceus* (Bull.) Fr.Cke., *C. comatus* (Fl. Dan.) Fr.Cke., *Lepiota cepaestipes* (Sow.) Fr., and *Armillaria mellea* (Vahl) of the Agaricaceae; *Cyathus striatus* (Huds.) Pers. and *Cyathus olla* (Batsch) Pers. of the Nidulariaceae; and *Bovistella radicata* (Mont.) Pat. and *Calvatia cyathiformis* (Bosc) Morg. of the Lycoperdaceae.

CULTURES.—Two types of cultures were used, namely, the Van Tieghem hanging drop culture and a petri dish type of culture. A detailed description of the technique employed in mounting and using the Van Tieghem cell is given by CLARK (6).

The petri dish technique was developed to facilitate the use of large numbers of cultures. Into each 100 mm. petri dish there was placed a piece of filter paper 9 cm. in diameter, moistened with the particular culture solution to be employed. The culture drops were disposed each on a cover slip, the spores were sown, and the cover slips arranged on the moist filter paper, covered, and incubated. For microscopic examination of the spores in the drop the cover slip was aseptically removed from the petri dish and inverted over a mounted glass ring. When in use, the petri dishes were kept in metal cans each provided with a convenient basket for removal of the dishes and moist paper towelling for maintaining humidity.

SOWING OF SPORES.—To minimize clumping tendencies, all spore suspensions were made in a physiological salt solution consisting of: 3.0 gm. sodium chloride, 0.2 gm. anhydrous calcium chloride, and 0.2 gm. potassium chloride to a liter of distilled water. Spores were transferred to this from the spore print with a platinum loop in the proportion of one loopful of spores to every cubic centimeter of the salt solution.

In suspending the spores of the Nidulariaceae, the peridioles were sterilized for 10 minutes in 20 per cent calcium hypochlorite, washed off with sterile distilled water, transferred to the sterile salt solution, and there crushed against the inner walls of the test-tubes with a sterile glass rod, thus liberating the spores.

Transfer of the suspension to culture drops was made with capillary pipettes, each flanged at one end to fit snugly into a rubber nipple and drawn out at the other end to a diameter of 250 μ and a length of 8 cm. This size delivers spores in such quantities as to provide an average of about 60 spores to the microscopic field of the high-power objective. Transfer of culture solution to cover slips was made with pipettes 15 cm. long and fire polished at one end to deliver small drops of uniform size and shape.

GENERAL TECHNIQUE.—All of the preceding operations were conducted inside a large transfer chamber, which was thoroughly sponged on the inner sides with a strong mercuric bichloride solution before being used. As a precaution against dust, a constant supply of steam was provided by boiling water inside the chamber. In addi-

tion, the usual bacteriological precautions were rigorously observed and no difficulty with contaminations was experienced.

Unless otherwise stated, incubation was in the dark and at a constant temperature of 28° C.

All glassware was cleansed with special care, first being boiled in a weak ammoniacal solution, then in a sulphuric acid-chromate solution, and finally in distilled water. Sterilization of dry glassware was accomplished by a two-hour exposure in a hot air oven at 170° C.

TABLE I

SPORE GERMINATION IN A STANDARD NUTRIENT SALT SOLUTION OF PH 4.6

Incubation in hours	Total no. of spores	Germination			Length of germ tube	
		No.	Percentage	Average (per cent)	Microns	Average in microns
288	29	2	9.09	8.16	19.47	14.75
	17	1	5.88		17.70	
	42	4	9.52		7.08	
384	40	2	5.00	7.95	42.48	51.92
	19	2	10.52		10.62	
	48	4	8.33		102.66	
508	55	5	9.09	11.75	10.62	12.98
	52	5	3.84		14.16	
	72	16	22.22		14.16	
Average	.....	.....	.....	9.28	.....	26.55

In making observations of the germination in drop cultures, three readings were taken at each inspection, and measurements of the lengths of germ tubes were made with a high-power objective and an ocular micrometer. Each reading was taken from a definitely different field of the drop and consisted of observing the total number of spores, the number of germinated spores, and the length of a germ tube taken at random. In general, the whole procedure is more readily exhibited by a sample protocol (table I), giving the germination of the spores of *Coprinus micaceus* in one of the 19 general culture solutions.

A spore was considered germinated when a germ tube of at least

1.77 μ had formed, this length corresponding to one-half unit of the scale on the ocular micrometer used. All germination percentages given in the results are actual. The practice of expressing the highest percentages as 100 per cent and adjusting other results accordingly was avoided in this investigation.

Investigation

I. Germination of spores of certain Agaricaceae and Nidulariaceae

Nineteen culture solutions were first tested for their value in stimulating germination of the spores of *Coprinus micaceus* and *C. comatus*. Of these media, three were selected as best suited for investigating the effect of hydrogen-ion concentration upon spore germination. Then the influence of temperature was determined, using the most effective medium at optimum hydrogen-ion concentration for the germination of the spores of each of the six organisms used.

A. Spores of Coprinus micaceus and C. comatus in the nineteen media.—The three culture solutions selected for further germination studies are the 0.5 per cent glycerin solution, the 4 per cent sucrose solution, and the beef broth. The reasons for this selection are apparent from a study of the results secured during an incubation period of 672 hours and are recorded in table II. The beef broth solution and the 4 per cent sucrose solution yielded the highest germination percentages for the spores of *Coprinus micaceus;* and the 0.5 per cent glycerin solution and 4 per cent sucrose solution, the highest for the spores of *C. comatus*.

Drawing conclusions from results secured with 19 solutions of varying strengths and of varying hydrogen-ion concentrations is unwise, yet one tendency which seems to be indicated in this preliminary test must be mentioned, that hydrogen-ion concentrations between pH 5.9 and 7.7 appear to favor germination of the spores of *Coprinus micaceus;* and concentrations between pH 5.9 and 6.7, germination of the spores of *C. comatus*.

B. Effects of hydrogen-ion concentration on six species of spores.—A series of 15 solutions ranging in hydrogen-ion concentration from pH 3.0 to 10.0 was prepared from each of the three culture

solutions selected: the beef broth solution, the 4 per cent sucrose solution, and the 0.5 per cent glycerin solution. The series was prepared by the use of determined quantities of N/5 H_3PO_4 and N/10 NaOH. The petri dish method was employed in order to accommodate this large number of tests.

TABLE II

SUMMARY OF GERMINATION PERCENTAGES AND GERM TUBE GROWTH OF SPORES OF COPRINUS MICACEUS AND OF C. COMATUS CULTURED IN GENERAL CULTURE SOLUTIONS*

Culture solutions	pH	C. micaceus		C. comatus	
		Per-centage	Tube growth in microns	Per-centage	Tube growth in microns
Nutrient solution	4.6	9.28	26.55	0.00	0.00
Beef broth	6.9	26.60	44.65	2.40	19.47
2% peptone	7.7	22.82	51.40	0.00	0.00
0.5% egg albumin	6.5	22.08	35.84	0.00	0.00
2% glucose	5.9	17.24	37.02	7.64	97.99
4% sucrose	5.9	27.23	43.41	10.11	30.85
0.5% glycerin	6.7	22.17	41.44	14.62	25.87
1% potato starch	6.4	16.36	34.93	0.00	0.00
Acetic acid	3.7	12.13	96.72	0.00	0.00
Oxalic acid	3.3	8.71	48.38	0.00	0.00
Citric acid	3.5	3.47	20.94	2.30	46.12
Hydrochloric acid	2.9	2.47	37.85	0.00	0.00
0.5% ammonium tartrate	6.3	15.81	32.92	2.62	12.53
0.5% calcium phosphate	6.5	17.62	36.87	6.73	43.66
0.5% sodium carbonate	10.0	8.59	21.41	6.11	101.63

* No germination whatever occurred in tartaric acid, pH 3.4; glutamic acid, pH 3.1; sulphanilic acid, pH 2.3; and sulphuric acid, pH 2.9.

The results secured, summarized briefly in table III and graphically by figure 1, indicate at least four types of spore germination responses to variations in the hydrogen-ion concentration of the culture solution.

First, with the exception of the spores of the two species of *Coprinus*, the hydrogen-ion range in which germination of the spores of the Agaricaceae and Nidulariaceae will occur, under the conditions of these experiments, is between pH 5.0 and 8.5. In the case of *Cyathus olla* the range is even narrower, germination having occurred only between pH 6.0 and 8.5. As noted, the spores of *Coprinus co-*

TABLE III

SUMMARY OF GERMINATION PERCENTAGES AND GERM TUBE GROWTH OF SPORES OF SIX SPECIES IN THREE SERIES OF CULTURE SOLUTIONS RANGING IN HYDROGEN-ION CONCENTRATION FROM PH 3.0 TO 10.0

	Beef broth		0.4% sucrose		0.5% glycerin		Beef broth		0.4% sucrose		0.5% glycerin	
pH	Per-centage	Tube growth (μ)	Per-centage	Tube growth (μ)	Per-centage	Tube growth (μ)	Per-centage	Tube growth (μ)	Per-centage	Tube growth (μ)	Per-centage	Tube growth (μ)
	Coprinus micaceus						Coprinus comatus					
3.0	0.00	.....	0.00	.....	0.00	37.37	0.00	.....	0.00	.....	4.13	27.73
3.5	0.00	.....	0.00	.....	5.11	44.05	0.00	.....	0.00	.....	4.72	30.09
4.0	0.00	.....	0.00	.....	5.26	44.45	0.00	.....	0.00	.....	6.08	31.86
4.5	11.87	20.53	0.00	.....	5.73	58.29	0.00	.....	0.00	.....	6.85	30.97
5.0	12.18	42.88	0.00	.....	7.88	167.86	0.00	.....	0.00	.....	7.77	41.30
5.5	8.89	149.11	0.00	.....	13.48	186.05	0.00	.....	0.00	.....	12.26	44.30
6.0	17.25	178.88	0.00	.....	17.58	212.40	5.59	15.34	3.54	14.45	18.17	56.98
6.5	18.81	71.51	8.14	50.74	19.03	212.40	7.50	26.55	12.22	24.48	22.15	74.04
7.0	27.66	210.67	17.10	67.85	28.93	194.70	11.97	34.21	4.69	18.58	23.03	207.38
7.5	31.89	212.40	6.84	30.97	17.29	167.56	6.50	29.20	4.32	15.04	35.40	212.40
8.0	29.12	212.40	6.89	27.14	15.60	167.56	1.87	9.44	0.00	.....	31.45	155.40
8.5	13.71	176.25	4.35	25.57	14.66	97.15	0.00	.....	0.00	.....	26.65	122.13
9.0	10.77	106.64	2.26	21.83	15.44	77.49	0.00	.....	0.00	.....	23.62	114.16
9.5	15.30	75.05	2.38	11.21	13.79	43.27	0.00	.....	0.00	.....	13.27	53.39
10.0	10.47	25.07	1.93	9.44	5.63	28.32	0.00	.....	0.00	.....	6.39	25.66
	Lepiota cepaestipes						Armillaria mellea					
5.0	4.75	13.27	0.00	.....	0.00	.....	7.61	14.76	5.59	11.01	2.32	7.38
5.5	5.97	24.19	3.75	12.98	0.00	.....	9.80	20.65	6.68	10.62	2.01	6.29
6.0	9.09	24.39	4.73	16.91	2.96	8.86	6.44	57.82	8.56	23.45	4.02	9.44
6.5	11.60	53.10	6.00	22.71	4.49	12.98	6.43	28.81	10.99	15.04	4.57	10.03
7.0	11.71	33.51	4.66	23.89	4.86	20.35	8.57	20.26	4.57	15.04	6.53	13.57
7.5	0.00	.....	4.30	14.16	6.68	27.14	0.00	.....	5.45	12.39	5.43	12.98
8.0	0.00	.....	3.44	12.29	5.27	22.03	0.00	.....	4.36	11.06	3.16	10.89
8.5	0.00	.....	0.00	.....	5.45	21.83	0.00	.....	0.00	.....	1.90	10.62
	Cyathus olla						Cyathus striatus					
5.0	0.00	.....	0.00	.....	0.00	.....	0.00	.....	0.00	.....	2.66	13.27
5.5	0.00	.....	0.00	.....	0.00	.....	0.00	.....	4.48	16.52	4.21	21.83
6.0	0.00	.....	3.28	6.09	4.84	18.29	0.00	.....	5.98	24.32	5.15	31.47
6.5	5.97	43.07	4.41	9.05	5.34	31.27	7.24	48.38	8.09	25.37	6.05	36.87
7.0	9.23	77.88	4.34	29.20	4.84	21.83	6.94	33.82	8.42	34.81	8.62	64.90
7.5	7.92	62.77	5.28	43.61	4.04	15.14	5.40	55.46	7.58	22.42	8.69	46.61
8.0	16.24	56.64	3.57	16.52	3.56	10.62	0.00	.....	6.04	18.88	7.35	37.37
8.5	0.00	.....	2.78	14.16	0.00	.....	0.00	.....	0.00	.....	5.32	27.14

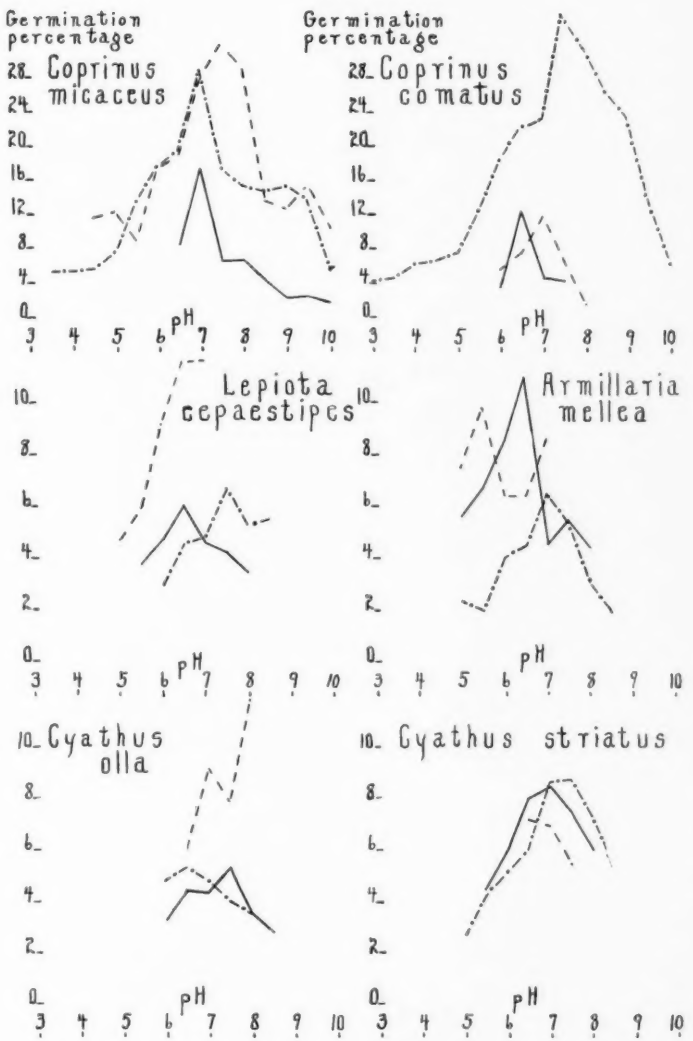

FIG. 1.—Effect of hydrogen-ion concentration upon germination percentage of spores of the six species cultured in beef broth, 4 per cent sucrose, and 0.5 per cent glycerin solutions: beef broth _ _ _ _; 4 per cent sucrose ——; 0.5 per cent glycerin _·_·_ .

matus germinated at all pH values in glycerin, and the spores of *C. micaceus* in all except pH 3.0.

Second, the general optimum pH for germination of the spores of the Agaricaceae and of the Nidulariaceae is indicated in these studies as 7.5. It is a little lower for two forms, namely, pH 7.0 for the spores of *Lepiota cepaestipes* and pH 6.5 for the spores of *Armillaria mellea*. Offsetting these, however, is the slightly higher pH value at which the spores of *Cyathus olla* germinate best, pH 8.0.

Third, the effect of the hydrogen ions and the hydroxyl ions upon spore germination varies. Germination of the spores of the two species of *Coprinus* and of the spores of *Cyathus olla* was more extensive on the alkaline side, and therefore can be said to have been favored by an excess of hydroxyl ions over hydrogen ions and somewhat inhibited by the opposite. Just the reverse is true of the germination of the spores of the other species; hence it appears that the effect of hydrogen ions or of hydroxyl ions upon germination of mushroom spores varies with the species.

Fourth, with the exception of *Coprinus*, the optimum hydrogen-ion concentration for germ tube development is in general slightly below the hydrogen-ion concentration optimum for germination.

Of the media used in this study the following were selected as being most favorable for spore germination: pH 7.5 beef broth for the spores of *C. micaceus;* pH 7.5 0.5 per cent glycerin for the spores of *C. comatus;* pH 7.0 beef broth for the spores of *L. cepaestipes;* pH 6.5 sucrose for the spores of *A. mellea;* pH 8.0 beef broth for the spores of *C. olla;* pH 7.5 0.5 per cent glycerin for the spores of *C. striatus*.

C. EFFECTS OF TEMPERATURE ON SIX SPECIES OF SPORES.—Spores of the same six species of the Basidiomycetae used in the hydrogen-ion tests were also used to determine the effect of temperature upon spore germination. The spores were cultured in the solution found best suited as a result of the preceding hydrogen-ion tests. Incubation was at ten temperatures ranging from 0° to 45° C. and arranged for by the use of a series of electrically controlled refrigerators and incubators. Lack of time made it impossible to examine each culture more than three times. Hence the results secured are lower than those in the hydrogen-ion test, but just as reliable, since

results of the hydrogen-ion test developed within 196 hours, the incubation period allowed for the temperature tests.

The results secured are summarized briefly in table IV, graphically by figure 2, and indicate the following tendencies.

The general optimum temperature for the germination of the spores under the conditions of this investigation can safely be stated as 30° C., the one exception being that of *C. micaceus*, with a maximum in these tests at 35° C. It is evident from this that higher germination percentages would have been secured by others investigat-

TABLE IV

SUMMARY OF GERMINATION PERCENTAGES AND GERM TUBE GROWTH OF SPORES OF THE SIX SPECIES CULTURED AT SEVEN DIFFERENT TEMPERATURES IN THE SOLUTION FOUND BEST FOR EACH SPECIES

Temperature (°C.)	Coprinus micaceus		Coprinus comatus		Lepiota cepaestipes		Armillaria mellea		Cyathus olla		Cyathus striatus	
	Percentage	Tube growth (μ)	Percentage	Tube growth (μ)	Percentage	Tube growth (μ)	Percentage	Tube growth (μ)	Percentage	Tube growth (μ)	Percentage	Tube growth (μ)
15	1.72	212.40	3.37	33.63	0.00	.....	0.00	.....	0.00	.....	0.00	.....
20	3.30	23.99	6.72	66.08	0.00	.....	0.00	.....	2.59	8.45	1.99	10.82
25	15.09	69.33	12.69	87.02	3.23	10.42	3.39	11.41	4.26	10.12	3.93	18.08
30	15.05	113.18	17.18	171.98	8.10	34.22	8.97	30.68	10.52	31.47	7.65	44.05
35	77.73	212.40	16.29	189.09	5.12	27.73	2.71	7.28	4.53	16.32	3.19	18.09
40	39.54	212.40	14.38	97.05	2.54	8.26	0.00	.....	0.00	.....	0.00	.....
45	27.48	156.94	7.58	44.84	0.00	.....	0.00	.....	0.00	.....	0.00	.....

ing spore germination had incubation temperatures been raised above the more popular 28° or 30° C., at least for species of *Coprinus*.

As to the temperature range, the widest adaptability was that manifested by the spores of *Coprinus*, which germinated at from 15° to 45° C. The narrowest range was that of the spores of *Armillaria mellea*, which did not germinate at a temperature below 25° C. nor at one above 35° C.

The temperature best for highest germination percentage is generally also optimum for longest germ tube development. The exception to this appears with the spores of the species of *Coprinus*, which developed longest germ tubes at a temperature 5° higher than the optimum for greatest germination percentage.

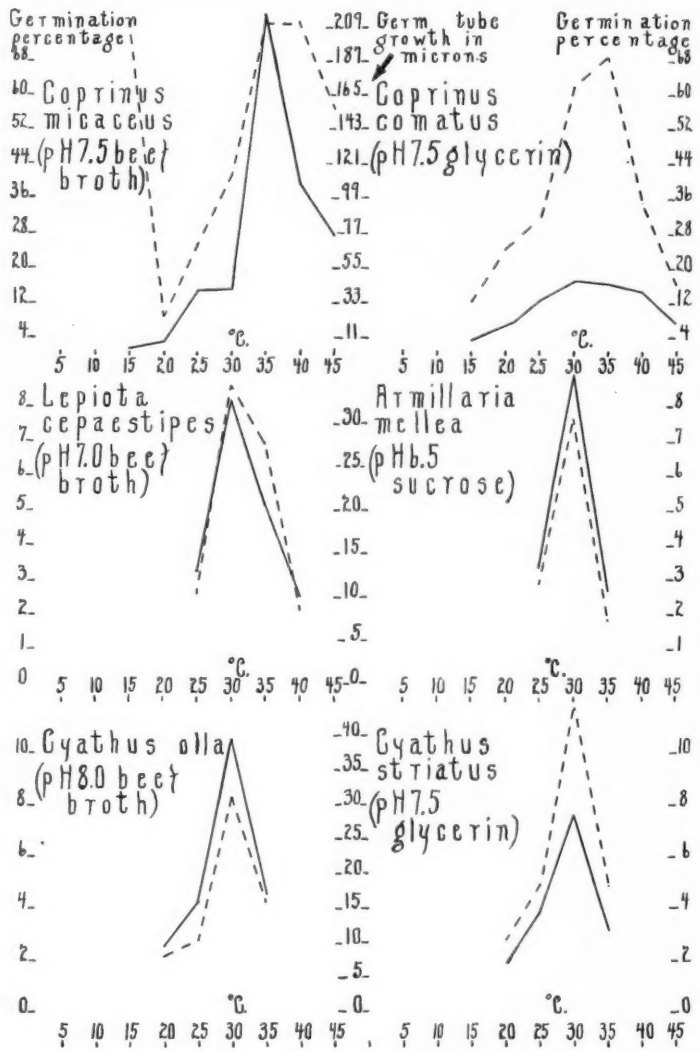
Germination percentage
Coprinus micaceus (pH 7.5 beef broth)
Germ tube growth in microns
Coprinus comatus (pH 7.5 glycerin)
Germination percentage
Lepiota cepaestipes (pH 7.0 beef broth)
Armillaria mellea (pH 6.5 sucrose)
Cyathus olla (pH 8.0 beef broth)
Cyathus striatus (pH 7.5 glycerin)
°C.

FIG. 2.—Effect of temperature upon germination percentage and upon germ tube growth of spores of the six species in the solution indicated for each: germination percentage ————; germ tube growth — — — —.

II. Germination experiments with spores of certain Lycoperdaceae

A. Spores of Bovistella radicata and Calvatia cyathiformis.—Of the 25 culture solutions used, 19 were the same as those used in the preliminary test of the first part of this paper. The other six solutions were weak infusions of well rotted pig dung, horse dung, and sheep dung, and weak infusions of sludge, silt, and Vigoro. Vigoro is a commercial preparation of 4.0 per cent nitrogen, 12.0 per cent available phosphoric acid, and 4.0 per cent water-soluble potash.

Despite an incubation period of 1200 hours for all solutions, no germination whatever was observed. Hence it was thought advisable to restrict further investigation to just one species of the Lycoperdaceae. The spores of *Bovistella radicata* were selected because a greater supply of them had been collected than of other forms.

B. Exposure of spores of Bovistella radicata to "natural" conditions.—Of the conditions probably prevailing about these spores as they germinate in nature, those tested were alternate wetting and drying as suggested by Swartz (**18**), alternate freezing and thawing, and exposure to extreme humidity.

Alternate wetting and drying of spores was accomplished with three petri dish cultures having ten cover slips each and a drop of distilled water, inoculated with the spores, on each cover slip. One of these cultures was incubated at 25°, a second at 30°, and a third at 34° C. The drops evaporated very rapidly and were replaced with sterile distilled water daily. This was continued for 17 days. Beginning with the eighth day, however, a cover slip with its inoculated drop was removed from each petri dish, transferred to a Van Tieghem cell, and incubated at 28° C. This was repeated daily until all 30 cover slips had been transferred to Van Tieghem cells. No germination occurred during the time allowed, 792 hours.

Spores were given freezing and thawing treatments by daily exposures of 4 hours to low temperatures ranging from −18° C. at the beginning of the period to 0° C. at the conclusion. At other times these spores were kept at 27° C. This procedure was followed on each of 8 days, when the spores were cultured in distilled water, nutrient salt solution, 7 per cent sucrose pig dung infusion, and physiological

salt solution. Germination did not occur even after 672 hours of incubation.

Spores were subjected to conditions of extreme humidity by seeding them on cover slips placed in the lower shells of petri dishes that were supported over water in a humidity chamber. But after 826 hours of this type of exposure no traces of germination had appeared.

C. EXPOSURE OF SPORES OF BOVISTELLA RADICATA TO AGENTS INTENDED TO INCREASE SPORE MEMBRANE PERMEABILITY.—This third phase was based on the possibility that delayed germination of these spores may primarily be due to impermeability of the spore membranes to stimulative and nutritive substances. Efforts to overcome this impermeability consisted of exposing the spores to change of osmotic pressure, low temperature, high temperature, enzymes, anaesthetization, and various combinations of these.

Exposing the spores to greater osmotic pressure was accomplished by culturing them in drops of the six infusions to each of which 7 per cent sucrose had been added. Germination in these solutions did not occur even after an incubation period of 936 hours.

Very low temperature was made possible through the use of liquid air secured in a thermos flask into which hard glass test-tubes containing spore masses were lowered successively for the varying periods of 2, 5, 10, 30, and 60 minutes. Following these exposures the spores were cultured in distilled water, physiological salt solution, nutrient salt solution, 7 per cent sucrose pig dung infusion, 7 per cent sucrose horse dung infusion, Troemmer's malt extract solution containing enzymes from a 7 months' old culture of *Aspergillus niger*, and nutrient salt solution containing enzymes from a 7 days' old culture of *A. niger*. An incubation period of 816 hours was allowed but germination did not occur.

Two methods were undertaken to test the effect of high temperature upon the germination of the spores. One was to incubate the spores in culture media at temperatures of 30°, 34°, 38°, and 42° C. The media used were the 2 per cent glucose solution and the oxalic acid solution. Spore germination did not occur during an incubation period of 1320 hours.

The other method was to expose spores to 100° C. for periods of 5 and 10 minutes. Moreover, the 5 minutes' exposure to 100° C. was

given the spores which had previously received the alternate thawing and freezing treatment, the spores which had been exposed to the temperature of liquid air for 10 minutes, and those which had been exposed to the temperature of liquid air for 30 minutes. Further, these spores were then cultured in distilled water, in 7 per cent sucrose horse dung infusion, and in young enzyme solution. This combination of treatments yielded no spore germination during an incubation period of 768 hours.

Ethylene chlorohydrin was selected for attempting to increase spore membrane permeability by anaesthetization. The spores were treated in test-tubes. Exposures were of two periods, 15 minutes and 30 minutes, daubers holding cotton saturated with ethylene chlorohydrin being withdrawn at the end of these periods and replaced with cotton plugs. This treatment was also given spores which had previously been subjected to alternate thawing and freezing, spores which had been exposed to the temperature of liquid air for 10 and 30 minutes respectively, and spores which had been subjected both to the temperature of liquid air for 10 minutes and that of 100° C. for 5 minutes. These spores were then cultured in distilled water, in 7 per cent sucrose horse dung infusion, and in young enzyme solution. Some of the spores therefore received the combined effect of low temperature, high temperature, anaesthetization, and maceration by enzymes. However, germination did not occur during an incubation of 768 hours.

Summary

As a result of these researches, and granted the conditions of this investigation, the following conclusions seem justified.

1. Three culture solutions in which a high percentage of the spores of *Coprinus micaceus* and of *C. comatus* will germinate are beef broth, 4 per cent sucrose, and 0.5 per cent glycerin.

2. With the exception of the spores of the species of *Coprinus*, the hydrogen-ion range in which the germination of the spores of the Agaricaceae and Nidulariaceae will occur is between pH 5.0 and 8.5. The spores of *C. comatus* germinate over a range of from pH 3.0 to 10.0, and those of *C. micaceus* over a range of from pH 3.5 to 10.0.

3. The general optimum pH for germination of the spores of the Agaricaceae and of the Nidulariaceae is pH 7.5.

4. The effect of the hydrogen ions and of the hydroxyl ions upon the germination of the spores of the Agaricaceae and the Nidulariaceae varies with the species.

5. The optimum hydrogen-ion concentration for germ tube development is slightly below the hydrogen-ion concentration optimum for germination.

6. The general optimum temperature for the germination of the spores of the Agaricaceae and of the Nidulariaceae is 30° C.

7. The widest temperature range under which germination will occur is from 15° to 45° C. for the spores of *Coprinus micaceus* and of *C. comatus*. The general range is from 20° to 40° C. for the spores of the other species of Agaricaceae and Nidulariaceae.

8. Up to the time of this writing, efforts at germinating the spores of *Bovistella radicata* and of *Calvatia cyathiformis* in artificial media have been unsuccessful.

9. In this investigation, the germination percentages of the spores of *Coprinus comatus* are higher than all other results thus far reported for this species.

10. This is the first definite investigation reported of the germination of the spores of four of the species used, namely, *Lepiota cepaestipes*, *Armillaria mellea*, *Cyathus olla*, and *Cyathus striatus*.

The writer is indebted to DR. B. M. DUGGAR for his suggestion of this problem and for his kind and valuable advice during the progress of the investigation and preparation of this report.

DEPARTMENT OF BOTANY
VALPARAISO UNIVERSITY
VALPARAISO, INDIANA

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A STUDY OF THE VEGETATIVE PHASES OF EPHEDRA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 457

PAUL D. VOTH

(WITH TWENTY-FIVE FIGURES)

Introduction

According to STAPF (15), species of *Ephedra* are found in the drier, warmer regions of every continent except Australia. The genus has received, together with *Gnetum* and *Welwitschia*, much attention in phylogenetic studies. The value of certain Asiatic species of *Ephedra* as a source of ephedrine, and of other species growing in the arid southwestern part of the United States as browse for cattle, sheep, and goats (6), has increased the economic interest in the genus.

The germination of *Ephedra* has been described in some detail by STRASBURGER (17) and by BOWER (4). Both stress the fact that *Ephedra* differs from *Gnetum* and *Welwitschia* in that no lateral projection of the hypocotyl (foot) is present in the former. BOWER (4) postulates that the suspensor and the living remains of the nucellus may act as conductors of nutrient material from the megagametophytic tissue to the seedling. SCHACHT (14) as well as HILL and DE FRAINE (10) show *Ephedra* seedlings with a structure that appears to be a foot. MARKGRAF (12), although he illustrates some of the stages of germination after HILL and DE FRAINE, correctly pictures the seedling without a feeding organ.

STRASBURGER (17) was the first to describe the root-stem transition in the hypocotyl of *Ephedra*. He pointed out that the vascular bundles in the hypocotyl follow a course similar to that followed by the bundles in the hypocotyl of *Araucaria*. The only other description of the vascular system in the hypocotyl of *Ephedra* is given by HILL and DE FRAINE (10). In their illustration a semicircular girdle of transfusion tissue connects the two hypocotyledonary bundles at a distance considerably below the cotyledons. No such condition was found in the species under investigation.

Apparently no detailed studies have been made to determine the vascular situation in the epicotyl of an *Ephedra* seedling. The course of the vascular bundles in the stem tip and the larger branches of plants past the seedling stage has been the subject of investigation since the time of NÄGELI (13). His longitudinal diagram of the stem of *E. vulgaris* roughly agrees with the situation in the stem of *E. pedunculata*, the species upon which the greater part of the present paper is based. GEYLER (9) extended the observations of NÄGELI to *E. equisetiformis* Webb and Berth., to *E. helvetica* C. A. Meyer (*E. distachya* Gand.), and to *E. campylopoda* C. A. Meyer (*E. distachya* Durv.), all of which were in accord with the species described by NÄGELI. STRASBURGER (17, pp. 77–78) reported that *E. altissima* does not have the customary eight bundles in the stem but only six, owing to the non-divergence of a pair of bundles in the second internode below the node at which the bundles diverge into the leaves. The vascular bundles in the stem continue for two internodes before they diverge into the opposite leaves. DE BARY (1) reviewed the vascular condition in the different species of *Ephedra* and referred to the work of STRASBURGER (17) on *E. campylopoda*, who showed that a "complementary" bundle is differentiated parallel to and between the two foliar bundles in the internode just below the node at which the foliar bundles diverge into the leaves. The "complementary" bundle evidently is lost in the girdle of xylem which is found at every node. This explains the occurrence of ten bundles in the stem of this species.

The number of bundles in the stem of *Ephedra* has been made one of the diagnostic characteristics of a new Asiatic species described by STAPF (16). LIU (11) has since shown that the number (eight) mentioned by STAPF is not constant, and that as many as ten bundles are found in the older parts of the plant.

STRASBURGER (17) and BERTRAND (2) have compared the anatomical features of *Ephedra* and the other Gnetales with those of the Coniferales. EVANS (7) described the anatomical features of several North American species of *Ephedra*, emphasizing the xeromorphic structures of stem and leaf. More recently GEORGE (8) has concluded that anatomically *Ephedra* is gymnospermous in its affinities. COULTER and CHAMBERLAIN (5) have discussed some of the anatom-

ical features of the Gnetales in relation to phylogeny. BOODLE and WORSDELL (3) compared the anatomy of *Ephedra* with that of *Casuarina*.

Materials and methods

Seeds and vegetative portions of *Ephedra pedunculata* Englm. were collected about 8 miles southeast of Lubbock, Texas, in May and June, 1931 and 1932. The vegetative parts were preserved in a formalin-alcohol solution containing 6 per cent commercial formalin and 60 per cent of 95 per cent alcohol. The seeds with their surrounding red, fleshy involucral bracts were air dried and later germinated in the University of Chicago greenhouses.

Most phylogenetic studies on *Ephedra* have been made on plants old enough to have lost many of their primary features. This paper records some observations on the primary stelar condition of the seedling.

Stem tips of *E. viridis* Coville, also a North American species inhabiting the arid southwest, and of *E. altissima* Desf. (?), a North African species, were used for comparison.

Seedlings used for the study of the root-stem transition were 33 days old; those of the epicotyl, 51 days. Formalin-alcohol (6–60 per cent), Flemming's medium, or Sax's modification of Nawaschin's fluid were used as fixing agents. Serial sections were cut 12 μ thick.

Investigation

SEEDLING

Under favorable conditions the seeds germinate in about ten days. The hypocotyl elongates rapidly and in two to five days the primary root, devoid of laterals, has grown two or three inches. Concurrently the cotyledons elongate, especially in their basal portions. The seed coat either remains in the substratum and the two cotyledons (bent as a knee in their basal portion) emerge completely, or the seed coat is brought above ground by the cotyledons, their tips still in position to absorb food from the remaining tissue of the megagametophyte. After a short period of growth the upper part of the hypocotyl straightens out. In the greenhouse, in direct light, the cotyledons average 5 cm. in length and 1 mm. in diameter. They are linear in

outline and in cross-section are roughly semicircular, their flattened sides facing each other and the stem. The cotyledons function for a time as photosynthetic organs.

At about 15 mm. below the divergence of the cotyledons the axis is definitely recognizable as primary root. This portion of the axis is smaller in diameter than the hypocotyl. The cortex, which is soon crushed, becomes darkened.

The diameter of the axis is much greater at the level of the divergence of the cotyledons, the bases of which form a cotyledonary sheath about 1.5 mm. in length.

The leaves are opposite, in decussate arrangement. Near the growing point the internodes are very short, but when mature they average 3 cm. in length. During the early life of the seedling, when the primary root continues to grow and the lateral roots are formed, growth of the epicotyl is very slow; later the rate of elongation is largely dependent upon water supply.

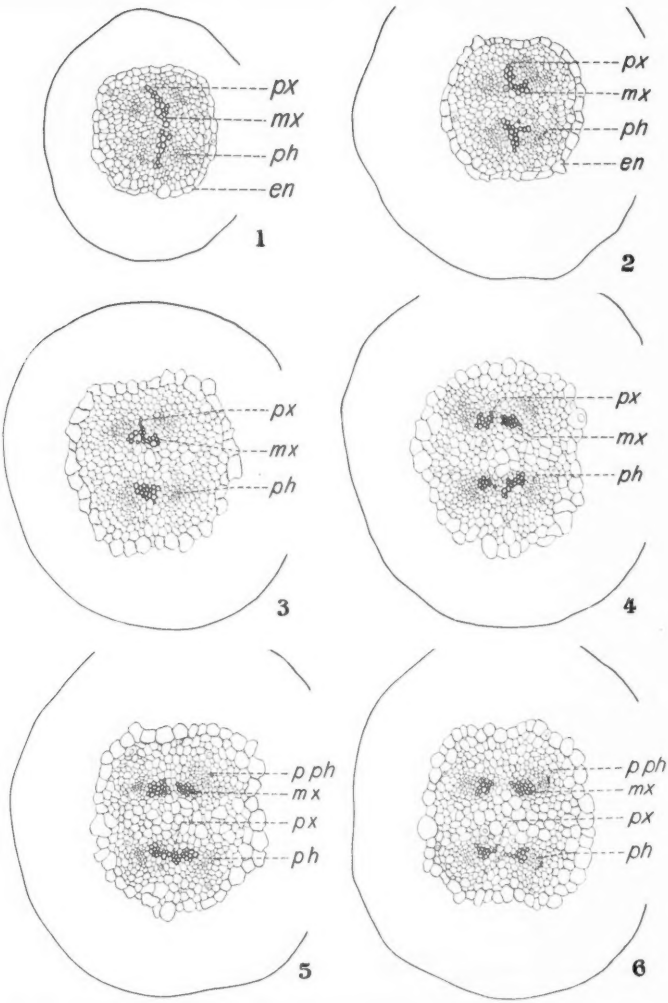
In the greenhouse the leaves of *Ephedra* remain functional for more than a year, but in the arid native habitat they become dry and scalelike within a few weeks. The stem functions as a photosynthetic organ until secondary growth and weathering disrupts the palisade layers in the cortex.

As will be seen from figures 21 and 22, buds can form in the axils of the cotyledons. Such buds, as well as those in the axils of the leaves, become active when the stem tip above them is clipped or grazed off. More than one bud may form in an axil, which produces the whorled appearance of branches of the older native plants.

Primary root and root-stem transition

The primary root is diarch, exarch. In some roots (fig. 1) a few cells in the center of the axis remain parenchymatous, in others the protostelic condition is attained only in the more distal portions.

In seedlings a month old the protoxylem elements are spiral or modifications of this general type. At places bordered pits occur on these elements. These pits are more numerous on walls where two tracheids are adjoined, but they also occur on walls which border on parenchyma cells. The walls of the metaxylem tracheids which are differentiated first (abaxial) are reticulate, while those differentiated



FIGS. 1–6.—Transverse sections of primary root and hypocotyl of a seedling 33 days old (only stele and endodermis shown in detail): Fig. 1, cross-section through primary root just below hypocotyl region, showing four phloem groups (*px*, protoxylem; *mx*, metaxylem; *ph*, phloem; *en*, endodermis). Figs. 2, 3, cross-sections through lower part of hypocotyl. Metaxylem is being differentiated abaxially. Figs. 4–6, cross-sections of hypocotyl showing tangential divergence of each primary xylem group (*p ph*, protophloem). ×47.

later are more heavily thickened and possess bordered pits with slit openings and tertiary thickenings. The innermost metaxylem tracheids do not mature until secondary growth has begun. At no level are more than four protoxylem elements visible at a protoxylem point.

The phloem of the primary root is difficult to distinguish. Several millimeters below the hypocotyl two phloem groups alternating with the two xylem groups are visible in cross-section. Near the hypocotyl four groups of elongated cells of small diameter, each with an elongated nucleus, are discernible lateral to the primary xylem groups (fig. 1); hence each xylem strand is flanked on either side by a phloem strand. No crushed protophloem is recognizable in the primary root.

A pericycle three or four cells in width, opposite the xylem groups and wider over the phloem, forms the periphery of the stele. At places this pericyclic zone of cells is ill-defined and merges into the parenchymatous rays which are at right angles to the length of the xylem groups.

At a level of 2 cm. below the divergence of the cotyledons (the pirmary root), the Casparian strips on the radial walls of the cells are so small that they are scarcely visible. At a higher level, in the hypocotyl, the Casparian strips are pronounced but are entirely unrecognizable at a level about 1 cm. below the divergence of the cotyledons. In general the endodermal cells are larger than the cells of the stele adjoining them, and are devoid of intercellular spaces.

The cortex of the root is usually less than six or seven cells in width. The cells are spherical with relatively small intercellular spaces. The epidermis of somewhat elongated tabular cells is soon sloughed off. Root hairs are found only rarely. With rapid growth of the root the endodermis often collapses early and the entire cortex sloughs off. No evidence of a periderm was found.

As indicated in figure 2, which is a cross-section 2 mm. higher than figure 1, the axis increases slightly in diameter. Viewed at this level the wedges of primary xylem appear more obtuse and farther apart. In reality the protoxylem points remain in the same position relative to the diameter of the axis, while cells more lateral and more abaxial to them become differentiated and mature into metaxylem.

At the same time a larger number of cells in the center of the axis remain parenchymatous and constitute the pith. At this level (fig. 2; also fig. 17, level 2′) and at all succeeding levels the axis is a dissected siphonostele.

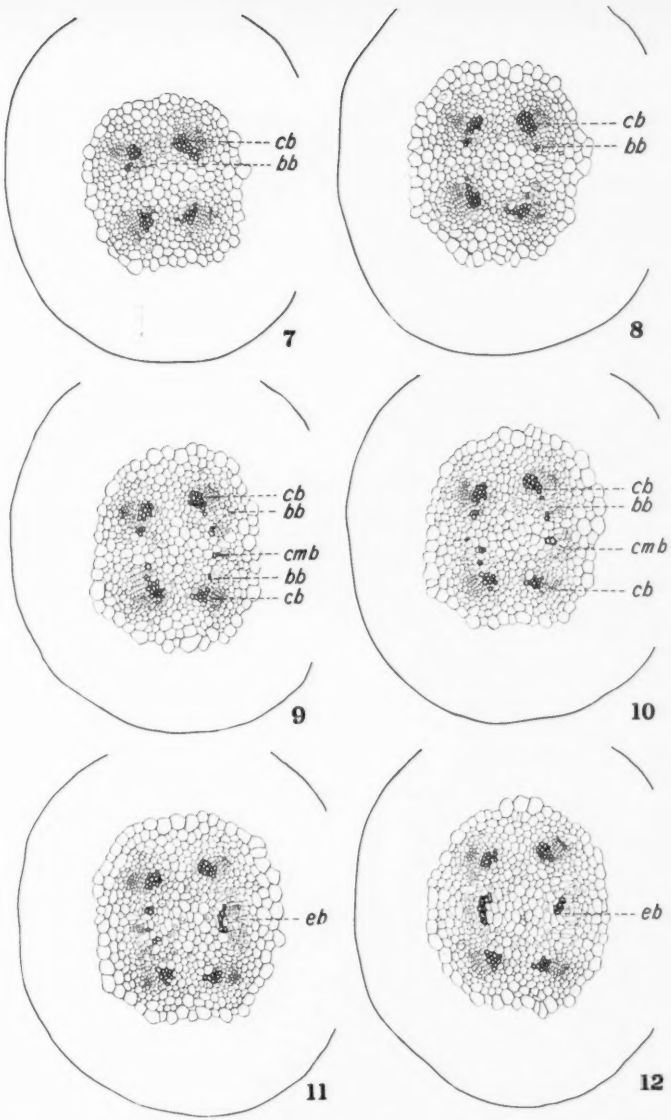
As shown in figure 3 (also fig. 17, level 3′), the metaxylem continues to be differentiated more centrifugally and tangentially higher in the hypocotyl, so that in cross-section each primary xylem group has the shape of an equilateral triangle with the protoxylem point exarch.

About 3.5 mm. below the divergence of the cotyledons (fig. 4), a section of the hypocotyl shows that each of the two exarch primary xylem strands becomes divided tangentially, appearing as a pair of xylem groups. These four strands of primary xylem continue into the cotyledons although they become endarch in arrangement; and each pair constitutes, together with their associated phloem groups, a cotyledonary trace. Casparian strips are best developed at this level. In all succeeding cross-sections the endodermis is doubtfully recognizable by its size, relative position, and the lack of intercellular spaces.

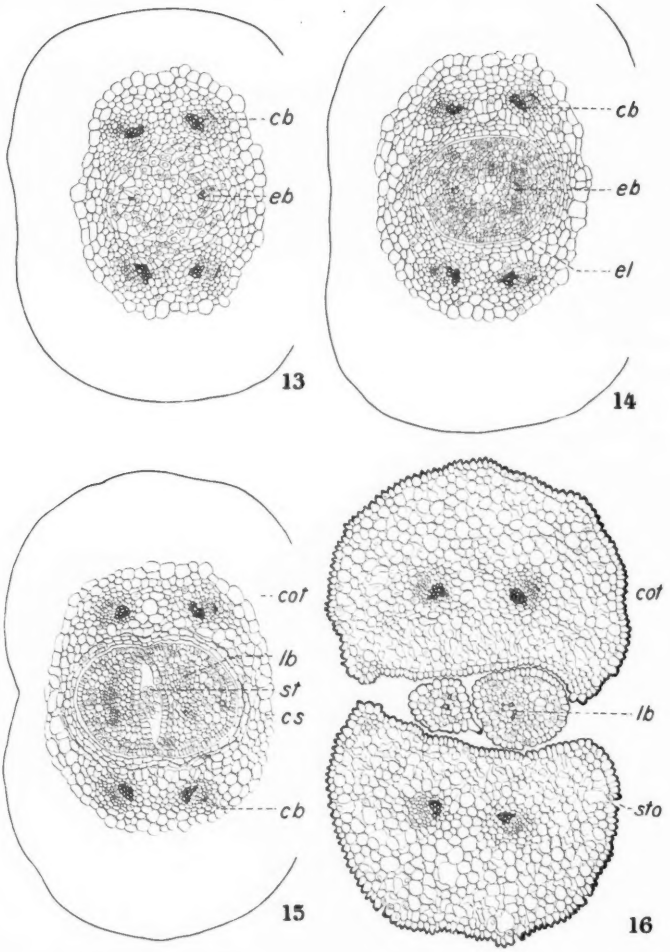
Cross-sections nearer the cotyledons show the following:

1. The metaxylem elements are differentiated in a progressively abaxial direction with reference to the protoxylem. At a level indicated by figure 7 (and fig. 17, level 7′) a condition exactly intermediate between exarch and endarch is shown. In succeedingly higher levels the centrifugal differentiation continues, so that when the cotyledonary trace enters the cotyledons the bundles are completely endarch.

2. At the level of cotyledon divergence each cotyledonary bundle gives rise to a branch bundle (fig. 7 and following figures). The branch bundles depart adaxially and slightly toward the outside of the axis, so that at a slightly higher level a pair from opposite cotyledons meet. Just before the bundles of each pair anastomose with one another, another bundle composed of a few tracheids is differentiated between them. This new bundle (figs. 9, 10, *cm b;* fig. 17, levels 9′, 10′), which is designated as a complementary bundle, arises *de novo* with no connection with other vascular tissue at a lower level. This short bundle anastomoses with the converging



FIGS. 7–12.—Transverse sections of hypocotyl of a seedling 33 days old: Figs. 7, 8, appearance of branch bundles (*bb*) from cotyledonary bundles (*cb*). Figs. 9, 10, appearance of complementary bundle (*cm b*) with no vascular connection at lower end. (In fig. 10 the lower right branch bundle has failed to mature at this level.) Figs. 11, 12, anastomosis of a pair of branch bundles with each other and with complementary bundle to form epicotyledonary bundle (*eb*). ×47.



FIGS. 13–16.—Transverse sections of epicotyl (*el*) and cotyledons of a seedling 33 days old: Fig. 13, divergence of abaxial surface of cotyledons (*cb*, cotyledonary bundle; *eb*, epicotyledonary bundle). Fig. 14, divergence of adaxial surface of cotyledons. Fig. 15, transection through tip of axis. Cotyledons (*cot*) have not diverged from each other at this level (*lb*, leaf bundle; *st*, stem tip; *cs*, cotyledonary sheath). Fig. 16, transverse section through cotyledons and tips of first foliage leaves (*sto*, stoma). ×47.

pair of branch bundles from opposite cotyledonary bundles to form a single epicotyledonary bundle (figs. 11, 12, *eb*). Each epicotyledonary bundle is therefore connected at its base to three bundles; two

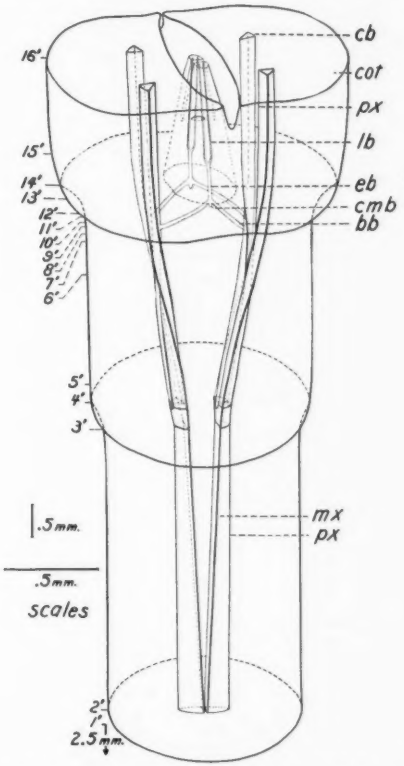


FIG. 17.—Schematic, longitudinal diagram of hypocotyl showing transition from root to cotyledons (drawn to scale with the levels of figs. 1–16 indicated as 1′ to 16′). Horizontal and vertical proportions are respectively three and one (*cb*, cotyledonary bundle; *px*, protoxylem; *mx*, metaxylem; *lb*, leaf bundle; *eb*, epicotyledonary bundle; *cm b*, complementary bundle; *bb*, branch bundle). ×47.

of these are tangential extensions from opposite cotyledonary bundles, and one is a free bundle, arising *de novo* between these two.

At a later age the epicotyledonary bundle becomes a wide crescen-

tic girdle of vascular tissue resulting from the maturation of additional metaxylem elements. From the two crescentic girdles of vascular tissue at each node (in the first node designated as epicotyledonary bundles) two pairs of bundles arise, while two pairs continue through this girdle into the next internode and depart into the succeedingly higher leaves (figs. 18–25).

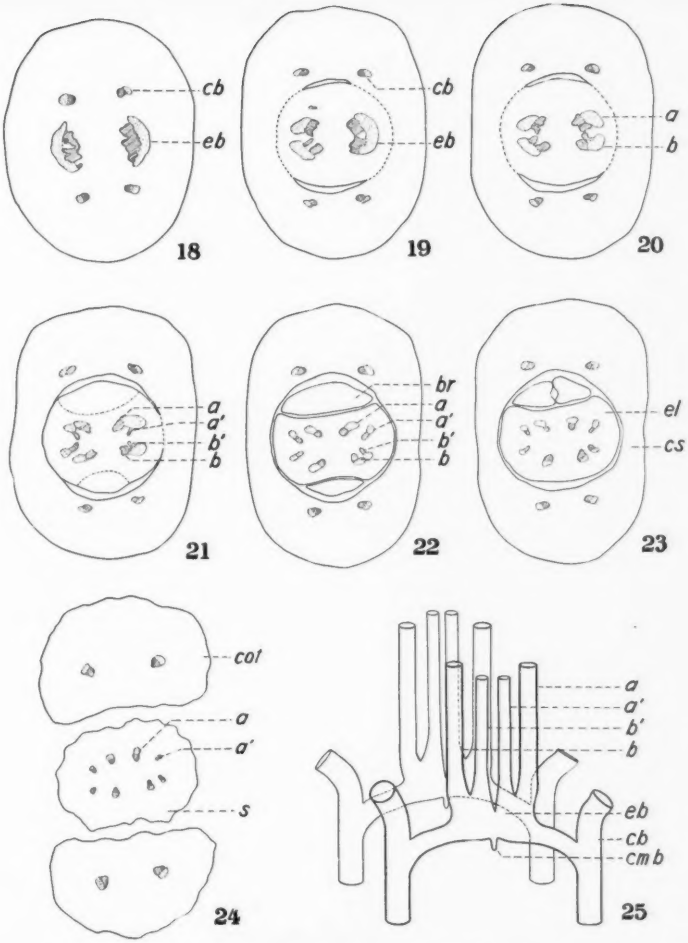
Cotyledons

The opposite cotyledons as well as the opposite leaves do not diverge from each other for some distance above the node, thus forming a sheath (figs. 14, 15). The flattened side of the semicircular cotyledons (as seen in cross-section) usually is concave, conforming to the curvature of the axis. The epidermis of elongated cells (four to five times as long as broad) is heavily cuticularized. In cross-section the radial and tangential dimensions of the cells are approximately the same. The stomata are sunken, the aperture communicating with the outside being guarded by four to six epidermal cells (7). Between the epidermis and the two vascular bundles of each cotyledon chlorenchymatous cells of various shapes are found. Those near the bundles are compact while those near the periphery have air spaces between them. Under each stoma the air space is relatively large. Near the base of the cotyledon the adaxial subepidermal cells are elongated palisade cells, with their long axis perpendicular to the axis of the foliage organ. In the upper end of the cotyledon (which emerged last from the seed coat) no definite palisade layer is recognizable. In the greenhouse the cotyledons function as photosynthetic organs for more than six months.

The phloem, which in the primary root was in four groups, two lateral to each of the two xylem groups, is differentiated abaxially at successively higher levels, so that in the cotyledons it is completely centrifugal with reference to the xylem. In the cotyledons the transition is complete; thus only the hypocotyl and the cotyledons have been directly involved.

Epicotyl and stem

At the age of 33 days only two decussate pairs of leaves have formed. The lower internode of these two shows some provascular



FIGS. 18–25.—Figs. 18–24, transverse sections at level of cotyledon divergence and through epicotyl of a seedling 51 days old. Figs. 18, 19, transverse sections at cotyledonary node. In fig. 19 the adaxial surface of the cotyledons is diverging from the axis and each epicotyledonary bundle (*eb*) is beginning to diverge at right angles to its length (*cb*, cotyledonary bundle). Figs. 20, 21, each epicotyledonary bundle (cresentic girdle) diverges equally to give rise to two bundles, *a* and *b*, which in turn diverge into *a*, *a'*, and *b'*, *b*. Fig. 22, cotyledons diverged completely from epicotyl. Buds in axils of cotyledons have given rise to branches (*br*). Figs. 23, 24, eight bundles in the stem differentiated so that they are nearly equidistant from one another (*el*, epicotyl; *cs*, cotyledonary sheath; *col*, cotyledon; *s*, stem). ×25.

Fig. 25.—Schematic diagram of vascular system in epicotyl and stem based on transverse sections shown as figs. 18 to 24 (*a*, *a'*, *b'*, *b*, as in figs. 21 and 22; *cm b*, complementary bundle). ×53.

tissue, the upper one none. No cell elongation has taken place. In the lower internode two opposite points, alternate in arrangement with the cotyledons, soon are sufficiently mature to be identified as protoxylem and protophloem elements and are continuous with the epicotyledonary bundle. At a higher level each one of these points branches equally, each pair of bundles constituting a leaf trace. In figure 16 the xylem elements in the first pair of leaves above the cotyledons are more mature than those in figure 15. Growth by cell division and cell enlargement brings the foliar bundles farther apart in the more distal portions of the leaf.

In cotyledons and leaves which are fully grown, the two foliar bundles run nearly parallel to each other and extend to within two or three cells from the epidermis of the tip. The few cells which intervene between the ends of these bundles later mature into short cells with reticulate thickenings, that is, transfusion tissue.

In seedlings which are 51 days old secondary growth has begun in the root and hypocotyl, but practically no cambium has formed in the axis above the cotyledons. Beginning with a section through the cotyledonary node comparable to figure 12, the primary tissues have matured more completely (fig. 18). Instead of the two small protoxylem points (fig. 13), two opposite crescentic girdles of primary xylem subtended by two collateral phloem groups are present. These girdles alternate in arrangement with the cotyledons and are comparable to the cotyledonary plate in other plants. The cotyledonary bundles have diverged at a lower level, as described in connection with the transition.

As the adaxial surface of the cotyledon departs from the axis, each cotyledonary girdle branches equally in the direction of its longest dimension. The direction of this divergence is alternate with that of the cotyledonary trace (fig. 19). Following so closely as to be almost concurrent, each of the four bundles just formed diverges in a plane parallel to the first divergence. Although not all of the eight bundles thus formed depart at the same level, a slightly higher section (fig. 20) shows that eight distinct bundles are present in the axis. In subsequent sections the bundles are differentiated in such a position that they are nearly equidistant from one another. The

two pairs of bundles which departed last are slightly smaller and roughly form the corners of a parallelogram which has its longest dimension at right angles to the cotyledons. These two pairs of bundles constitute the leaf traces for the succeeding node (fig. 25).

Stem tips of *Ephedra pedunculata* and of *E. viridis* were compared to determine the vascular condition of the node. Beginning at a level represented in figure 25, the pair of bundles at the narrow end of the parallelogram diverge into the leaves just before the node is attained. At the node the remaining six bundles are anastomosed into two semicircular girdles, much as the six bundles constitute the girdles (cotyledonary plate) in the first node. The crescentic girdles alternate in position with those of the preceding and succeeding nodes. From the girdles at each node eight bundles arise as described for the cotyledonary node (figs. 18–25). Such origin explains the presence of eight bundles in the stem of *Ephedra* as described by earlier investigators (**1, 9, 10, 13, 15, 17**). Variations from this number can be explained on the basis of the non-divergence of two pairs of bundles and by the differentiation of a "complementary" bundle between the bundles which form the leaf trace for the succeeding node (**17**).

Ephedra viridis possesses eight distinct bundles in its stem but the lateral differentiation of metaxylem proceeds until a continuous cylinder of xylem is formed, making it appear as though secondary growth began very early in the stem. *Ephedra altissima* (?) resembles *E. pedunculata* in the appearance of the internodal bundles.

Summary

1. The root-stem transition is described for *Ephedra pedunculata* Englm. and the vascular condition of the stem is compared with that of *E. viridis* Coville and *E. altissima* Desf.

2. The primary xylem in the root of *Ephedra* is diarch, exarch. The two groups of primary phloem alternate with the xylem.

3. The transition occurs in the hypocotyl. Each primary xylem group diverges into two lateral branches both of which continue into the cotyledon. Differentiation and maturation of the xylem elements in each cotyledonary bundle proceed in such a manner that

at successively higher levels the metaxylem becomes constantly more abaxial. The cotyledonary bundles are exarch when they depart into the cotyledons.

4. At the first node the cotyledonary bundles each give rise to a branch bundle. These bundles are differentiated toward each other in pairs. When these converging branch bundles anastomose with each other a third but shorter bundle joins them. The latter bundle arises *de novo*.

5. In an older stem the differentiation of additional metaxylem elements to the three bundles, forming an epicotyledonary bundle, gives rise to a crescentic girdle with its longest axis parallel to the leaves diverging at that node. From the pair of girdles at each node eight bundles arise and continue separately through the succeeding internode. Above the second node each bundle continues for two internodes before it departs into a leaf.

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STRUCTURAL RESPONSES TO THE PRACTICE OF TOPPING TOBACCO PLANTS: A STUDY OF CELL SIZE, CELL NUMBER, LEAF SIZE, AND VEINAGE OF LEAVES AT DIFFERENT LEVELS ON THE STALK

GEORGE S. AVERY, JR.

(WITH FOUR FIGURES)

Introduction

In the United States the common practice of producers of practically all but the shade-grown tobaccos is to go through the field a few weeks before the plants are mature and break off the terminal flower stalks. This practice, commonly referred to as topping, hastens the development of axillary branches, which are also removed on subsequent trips through the field. The latter procedure is usually referred to as suckering; but the plants mentioned in this study as topped actually were both topped and suckered. Such practices are in reality little more than the pruning so commonly resorted to for diverse reasons in the production of many plants of economic importance.

Normal growth of the tobacco leaf has already been considered (1), and topping and suckering practices as related to quality and yield have been discussed by others. The general result reported is an increased yield due to the stimulation of growth of the leaves at the upper end of the stalks of topped plants. Does this increase in leaf size mean that the practice of topping stimulates cell division and that the subsequent enlargement of these additional cells is responsible for the increased size, or does the increase come about entirely from the greater enlargement of existing cells? Do all tissues of the leaf necessarily respond to topping in the same manner? The principal purpose of this paper is to answer these questions and to discuss their possible bearing on the problem of the relation of function to differentiation of cells in certain tissues.

Materials and methods

The plants on which the following data are based were *Nicotiana tabacum* L. varieties Cash, Cuban Shade, and Havana Seed. There were six plants of each variety. The details recorded here are from the Cash variety, and are supported in entirety by less detailed observations on the other two. The seeds were planted in flats in the greenhouse in February and the seedling plants moved into 10 inch clay pots six weeks later. Growth continued over the ensuing 19 weeks, a much longer time than it would have extended under field conditions. Each leaf was allowed to remain on the stalk until fully mature, that is, until it started to turn yellow in color, but was removed before any wilting or shriveling took place. Half of the plants were topped at the 21st leaf on June 22, the other half retaining their flower stalks and flowers. The latter developed seeds (fig. 1).

The dates on which the first seven leaves (the so-called seed-bed leaves) were removed from the plants were not recorded, but the harvest dates of those from the 8th leaf upward are as follows for the untopped plants: 8th and 9th, June 12; 10th and 11th, June 14; 12th, June 20; 13th, June 22; 14th, June 26; 15th, June 28; 16th, July 2; 17th, July 10; 18th, July 13; 19th, 20th, and 21st, July 20. In the topped series, the increase in the duration of the growth period began to be apparent in the 16th leaf, and the dates of harvest from that leaf upward are as follows: 16th, July 13; 17th, July 20; 18th to 21st, August 23.

Samples about 1 cm. in diameter were punched from approximately the middle of the lamina on either side of the midrib of each leaf. These were preserved in formal-acetic-alcohol and were later used for determination of leaf thickness, veinage, cell size, cell number, etc. Leaf area was determined by cutting paper patterns to leaf size, then weighing. The samples used for tissue study were imbedded by the paraffin method and sectioned at a thickness of 12 μ. They were stained with safranin and fast green.

Determinations of cell size were made with the aid of a Leitz drawing ocular and a planimeter. Veinage determinations were made from the same leaf samples by heating them in a concentrated solution of chloral hydrate until clear, but even after this treatment the

thick upper leaves of the topped plants were difficult to work with. The veinage in a given microscopic field was drawn under low power

FIG. 1.—Effect of topping (photographs taken after leaves for comparative study had been removed): *a*, *b*, *c*, plants of Havana Seed, Cuban Shade, and Cash varieties, topped at the 21st leaf above cotyledons; *d*, *e*, *f*, same varieties, untopped (normal).

with the aid of a drawing ocular. Magnification and area of field were taken into consideration and the linear measure of veins was then expressed in millimeters per square centimeter of leaf lamina.

Leaves from bottom to top of stalk in normal and topped plants

AREA AND THICKNESS OF LEAVES

The gradually increasing size of the several successive leaves above the cotyledons is well understood, and in normal plants with 20 to 25 leaves (meant to include all the leaves ever formed on the plant) this increase ordinarily continues about half-way up the stalk. The next few leaves above this middle level are approximately the same size, and those toward the top are successively smaller. Plants with

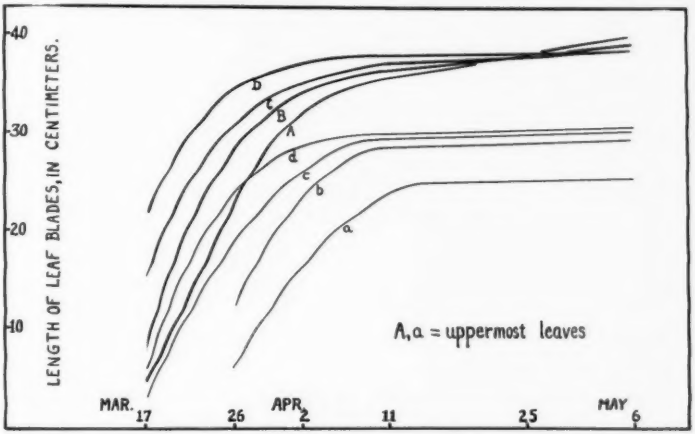

FIG. 2.—Differences in growth of upper four leaves of topped (*A-D*) and untopped (*a-d*) plants.

a greater number of leaves have a longer zone in which the leaves are of maximum size. In this experiment the 14th leaf was in the zone of greatest leaf size.

The upper leaves of the topped plants discussed here differ from those ordinarily produced under field conditions, both in their extreme enlargement in proportion to other leaves on the plant and in the fact that they are unusually thick and heavy-bodied to the touch. Topping has little or no effect on the leaves which are mature at the time of the operation, such as those on the lower half of the stalk; but at the time it is done several of the uppermost leaves are only partially developed, and it is these leaves which are stimulated to greater growth (fig. 2). The 17th, 19th, and 20th–21st leaves were chosen

as representative of the upper leaves of the plant. The 17th leaf shows little response to topping; the 19th shows a definite response; and the 20th–21st (averaged and computed as a single leaf) shows a marked response. Taken collectively, these leaves from topped plants average 32 per cent greater in size than corresponding leaves of untopped plants. The average thickness of the upper leaves of topped plants is as follows: 17th, 184 μ; 19th, 251 μ; 21st, 308 μ. Corresponding leaves from untopped plants average 188 μ, 200 μ, and 188 μ respectively.

Size of petiole and petiolar bundle

In untopped plants the transectional area of the petioles of successively higher leaves follows the trend of increasing size shown by the laminae of the same leaves, up to the middle of the stalk. Instead of decreasing in size from this level on toward the top, as does the leaf blade, the transectional area of successively higher petioles decreases little or none. In the corresponding petioles of topped plants there is an increase in transectional area which is roughly proportional to the increase in leaf size. Petiolar bundle size maintains a rather constant proportion to that of the petiole in both topped and untopped plants.

Cell number in various tissues

While topping does not bring about any observable change in numbers of cells in either epidermal or fundamental tissue, it stimulates secondary activity in the petiole and midrib of the upper leaves (fig. 3). As a consequence there is an appreciable amount of secondary xylem laid down (table I). Fewer lignified primary xylem elements differentiate in petioles of these upper leaves than in corresponding petioles of untopped plants, which suggests that the cambium is stimulated to activity relatively early. The cambium is increasingly active in successively higher leaves.

Cells of the internal pericycle have heavily thickened walls in leaves of both topped and untopped plants. They are easily distinguishable from sieve tubes and companion cells of the internal phloem groups, and the latter are easily distinguishable from other neighboring cells. The sieve tubes and companion cells occurring in

patches (both primary and secondary) were counted as the external phloem. The most careful counts possible on the number of cells of the external phloem parenchyma show no appreciable difference between topped and untopped plants.

It is a noteworthy fact that there are 20 per cent fewer sieve tubes and companion cells in the external phloem in petiolar bundles of the

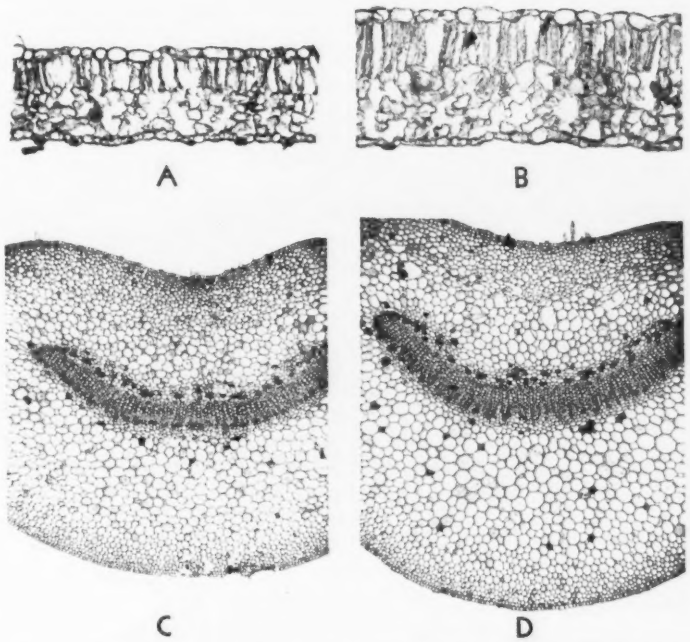

FIG. 3.—Photomicrographs of transections of blade and petiole of 20th leaf of normal and topped plants: *A*, *C*, untopped (normal) plant; *B*, *D*, topped plant.

21st leaves of topped plants than in corresponding leaves of untopped plants, a condition quite the opposite to the numbers of lignified xylem elements, despite cambial activity. The numbers of cells in the internal phloem (all primary) remain approximately constant from the 15th leaf upward to the 21st, while in the corresponding leaves of untopped plants the numbers increase sharply (table II).

Observations of ten of the smallest newly differentiated veins (21st leaves, topped plants) showed over 40 per cent fewer phloem parenchyma cells than corresponding veins in untopped plants.

TABLE I

NUMBERS OF PRIMARY AND SECONDARY XYLEM ELEMENTS AND THEIR AVERAGE TRANSECTIONAL AREA IN THE PETIOLAR BUNDLES OF 14TH–21ST LEAVES OF UNTOPPED (NORMAL) AND TOPPED PLANTS

Petiole of leaf no.	Untopped (normal)		Topped			
	Primary (metaxylem only)		Primary (metaxylem only)		Secondary	
	Average size in sq. mm.	Total no. of cells	Average size in sq. mm.	Total no. of cells	Average size in sq. mm.	Total no. of cells
14	0.00210	252	0.00217	.....	.....	.....
15	0.00157	276	0.00180	246	0.000826	27
17	0.00152	269	0.00131	201	0.000744	187
19	0.00140	299	0.00171	214	0.000573	293
21	0.00118	352	0.00153	223	0.000596	573

TABLE II

NUMBERS OF INTERNAL AND EXTERNAL PHLOEM ELEMENTS IN TRANSECTIONS OF PETIOLAR BUNDLES OF THE 17TH, 19TH, AND 21ST LEAVES OF UNTOPPED (NORMAL) AND TOPPED PLANTS

Petiole of leaf no.	Internal phloem		External phloem (primary and secondary)	
	Untopped	Topped	Untopped	Topped
17	255	287	382	364
19	283	294	360	392
21	330	293	411	344

Cell size in various tissues

The lignified primary xylem elements of the 19th and 21st leaves are larger in topped than in untopped plants (table I), which can be taken to mean only that the response to topping starts early, that is, even before the primary elements (metaxylem) mature. The second-

ary elements are very much smaller than the primary, and they decrease in size more sharply than the latter in the 16th to the 19th leaves.

The increase in size of cells in other tissues in response to topping is roughly proportional to the increase in the size of the leaf as a whole. The upper leaves (17, 19, 21) of topped plants as contrasted with the corresponding leaves of normal plants show the following: upper and lower epidermal cells together average 31 per cent greater in size; palisade parenchyma cells, 31 per cent; fundamental tissue cells in the petiole, 23 per cent greater (table III).

TABLE III

AVERAGE SIZE OF CELLS IN THE 17TH, 19TH, AND 21ST LEAVES OF UNTOPPED (NORMAL) AND TOPPED PLANTS. ALL FIGURES ARE IN SQ. MM., AND REPRESENT AN AVERAGE OF 25 CELLS EACH, EXCEPT FOR THE EPIDERMIS WHERE EACH FIGURE REPRESENTS AN AVERAGE OF APPROXIMATELY 25 CELLS EACH FROM BOTH UPPER AND LOWER

Leaf no.	Epidermis (upper and lower)		Palisade		"Cortical" cells of petiole	
	Untopped	Topped	Untopped	Topped	Untopped	Topped
17	0.00258	0.00238	0.00165	0.00175	0.1915	0.1920
19	0.00237	0.00318	0.00146	0.00168	0.1635	0.1610
21	0.00207	0.00363	0.00189	0.00315	0.1315	0.2475

These results compare with the 32 per cent greater size and the 29 per cent greater thickness of such leaves.

That the lower epidermal cells are consistently smaller than those of the upper epidermis in all the higher leaves is interpreted as meaning that cell division persists longer in this layer.

Vascular network and leaf size

Table IV illustrates the tendency for successively higher leaves of normal plants to have a greater "density" of veins (greater linear measure per unit area of leaf surface).

In the upper leaves of topped plants there is a tendency for veinage density to remain about the same from the 15th leaf upward, in spite of the fact that these leaves (17, 19, 21) average 32 per cent

greater in size. This means that additional veins continue to differentiate as the upper leaves of topped plants enlarge, and in sufficient numbers to maintain an almost constant proportion of veinage to leaf surface. Actually the laying down of new veins does not entirely keep pace with leaf enlargement (table IV and fig. 4).

Relative development of root and shoot in normal and topped plants

Root development of topped plants was markedly greater and the more robust stalks were equally characteristic (fig. 1). Anatomically

TABLE IV

INCREASED AREA AND DECREASED VEINAGE IN UPPER LEAVES OF TOPPED PLANTS AS CONTRASTED WITH CORRESPONDING LEAVES OF UNTOPPED (NORMAL) PLANTS

Leaf no.	Area in sq. cm.			Veinage (linear mm. of veins per sq. cm. of leaf surface)		
	Untopped	Topped	Percentage greater size topped leaf	Percentage less veinage in topped leaf	Untopped (normal)	Topped
14	209	.....	.....	.....	.....	.....
15	202	184	− 9.8	+13.3	626	709
17	184	194	5.4	6.2	696	742
19	188	263	40.0	4.4	757	724
21	166	254	51.0	9.4	798	723

the roots and stalks of topped plants show greater numbers of heavier walled xylem cells, a characteristic generally associated with plants which are high in carbohydrates.

Discussion

There seems to be little point here in trying to reconcile the multiplicity of results reported by different investigators, as to height of topping, yield, quality, etc. So much depends upon the nature of the soil on which the crop is grown, the fertilizer used, rainfall and other seasonal influences, as well as the ultimate use to which the tobacco is to be put, that the extent or degree of pruning (topping and

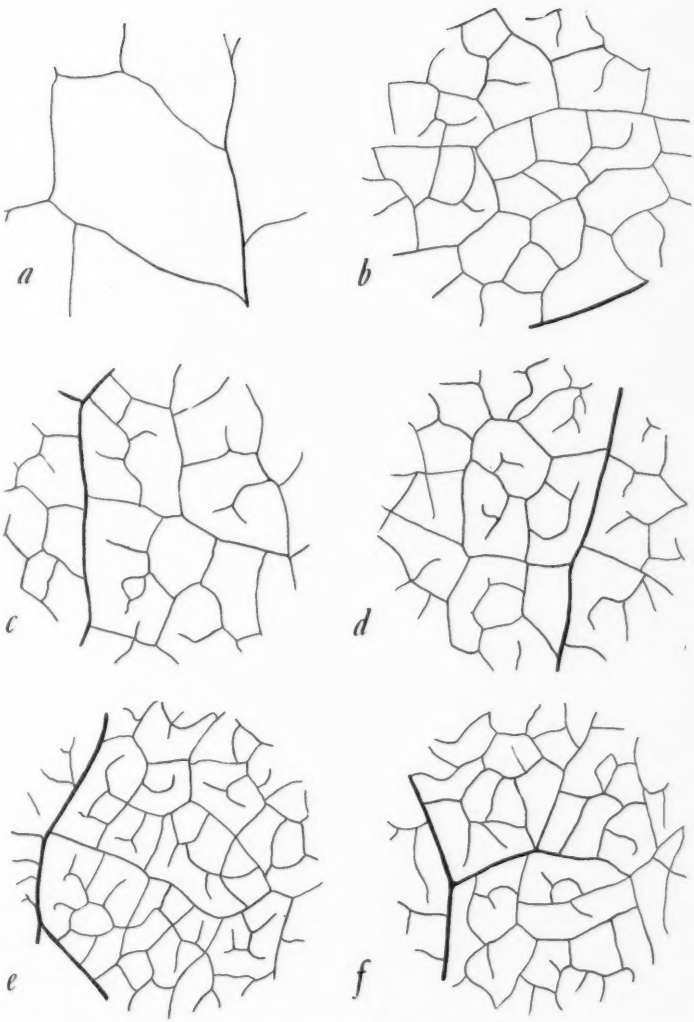

FIG. 4.—Portion of vascular network in cotyledon (*a*) and 4th (*b*), 8th (*c*), 13th (*d*), and 20th leaves above cotyledon. Network of 20th leaf is shown for both untopped (*e*) and topped plants (*f*). Variety Cash.

suckering) which is desirable depends largely upon the specific situation, a fact already well known by agriculturists.

There is, however, general agreement in the literature as to what may be expected from topped tobacco plants as contrasted with untopped plants: (a) They have a greater average leaf surface, depending to some extent on the number of leaves remaining on the stalk (**7, 13**); (b) the leaves are thicker (**7, 13**). It is reported that leaf thickness is in direct proportion to the number on the plant, that is, the fewer the thicker (**7**), or, as expressed by others, the fewer the leaves the coarser the "texture" (**3, 4**); (c) the growth response to topping is greatest in the upper leaves (**4, 7**); (d) the duration of the growth period is increased (**4, 7**), although one investigator later states (**5**) that topping accelerates the ripening of the leaves; (e) with increased leaf growth there is more extensive development of the root system (**4, 5, 7**); (f) the physiological explanation of the response to topping and suckering is reported (**3**) to lie in the removal of many of the users of carbohydrates, making it possible for the young leaves which remain to use most of the material synthesized by them for their own growth.

Such observations quickly resolve themselves into the question of so-called antagonism between vegetative growth and reproduction (**10, 11**). It has been shown that in certain annual plants with a determinate type of growth, increase in size of plant usually terminates soon after pollination. When the plants are defruited there is a renewal of vegetative development, or if the flowers are removed as rapidly as they open, the plants grow continuously at a uniform rate. The topping and suckering practices in tobacco, similarly, clearly stimulate further vegetative activity (fig. 2). Gross responses to topping substantiate the preceding previously reported investigations, but it is a curious fact that no detailed analysis has been made of the well known increase-in-size response shown by the upper leaves of topped plants.

Other comparable studies, with one exception, tend to bear out the validity of the results reported here. The removal of several of the buds and leaves from a given branch of *Tilia europaea* (**6**) did not result in excitation of renewed growth in the adult leaves. When done at a sufficiently early period in the development of the leaves,

however, they became abnormally large; such increased size was due to an increase in the number of cells. In another study (**18**) all apical meristems were removed from *Coleus* and *Phaseolus* plants and only a single leaf was allowed to remain on the stalk. This remaining leaf increased in size owing to an increase in the size of its cells. Comparable results have been reported for the behavior of cotyledons of seedlings (**19**): By removal of the main shoot as soon as it appeared above the cotyledons, further growth of the cotyledons took place, owing to an increase in the size of certain of their constituent cells. Another study (**15**) showed that in cotyledons which become hypertrophied early, the epidermal cells increased in size in almost exact proportion to the cotyledon itself; and in those which became hypertrophied later in their development, the increased size was due solely to further cell enlargement. Thus, whether concerned with cotyledons or the leaves which follow, a number of investigators have concluded that increase in leaf size, beyond certain limits, is due to a further increase in the size of already existing cells.

The greater-than-normal growth resulting from topping also finds a parallel in the growth of leaf cuttings of several different kinds of plants: Leaves may live longer when removed from stems and treated as cuttings (**8, 9, 14, 18**), and if properly cared for they continue to increase in area and thickness. This supplementary growth has been reported as due to an enlargement of existing cells (**8**), those of the mesophyll, epidermis, and ground parenchyma of the petiole having been observed to increase (**9**). It has been suggested (**14**) that the older leaves when left on the plant cease growing because of an inadequate water supply, owing to a diversion to the growing point, etc.

SCHUSTER (**17**) observed veinage development in leaf cuttings of several species which lived for periods varying from two months to three years. While there was a marked increase in size in the two-months-old cuttings, the veins apparently were merely stretched apart, and veinage ranged from 36 to 87 per cent normal. Those which grew for three years showed additionally differentiated veins, and veinage ranged from 121 of normal in *Hedera helix* to 173 per cent in *Aucuba japonica*. These new veins were reported as arising from dividing spongy parenchyma cells or from "conducting"

parenchyma. SCHUSTER concluded that while the proportion of veinage to leaf surface is apparently an inherent character in normal leaves, the proportion may change if functional demand is changed considerably; and further, that veinage is increased if the "nutrition" is increased. While SCHUSTER's use of "nutrition" is vague, there is a distinct parallel in the upper leaves of topped tobacco plants, where with the increased availability of mineral nutrients and foods there is also an increase in veinage.

It has been noted (**16**) that "in the great majority of Dicotyledonous plants the increase of xylem within the petiole accompanying increase in size of leaf is brought about by secondary thickening." Whether the laying down of this additional xylem in the upper leaves of topped plants is due to the stimulus of increased demands on the water supply, or whether it is the result of a change in the carbohydrate-nitrogen balance in the leaf, or both, is impossible to say. It is not improbable that a hormone may be involved. High carbohydrate accumulation is often associated with increased secondary thickening, however, and it might reasonably be expected that this same stimulus would promote the differentiation of additional vein network in the enlarging leaf blade.

ALEXANDROV and others (**2**) have observed that the water conducting system in the petioles of leaves of *Bryonia dioica* is correlated with the size of the leaf, and that the higher the leaf is situated on the stem the greater is the water conducting system leading to it, per unit of leaf surface. The present study bears this out for untopped (normal), but not for topped plants where size of the upper leaves is greater in proportion than is the increase in the water conducting system leading to them (32 and 22.3 per cent respectively).

Whatever the explanation, the evidence is clear that veinage is maintained in almost constant proportion to leaf surface. This suggests that the degree of development of the xylem and the amount of water loss are closely interdependent (**12**). But while secondary xylem cells differentiate in abundance following cambial activity in the upper petioles of topped plants, there is no further differentiation of secondary phloem in these same petiolar bundles. SCHWARZ (**18**) has observed an identical situation in leaf cuttings of *Coleus* but has not suggested an explanation. Any conclusion as to why the phloem

fails to increase appreciably, based on the evidence at hand, would be partly conjectural, and it seems sufficient to point out the correlation which exists: Phloem cells differentiate in untopped (normal) plants on which flowers and seeds develop in greater numbers than in topped plants where no flowers or seeds develop, even though the cambium is active only in the topped plants; that is, the absence of developing seeds and the subsequent cessation of diversion of material from leaves to the seeds (diversion of material to the root presumably continues) is accompanied by a cessation of differentiation of phloem cells.

Summary

1. In the tobacco plant all vegetative growth usually ceases soon after the seeds start to form. If the terminal flower stalk and all axillary branches are removed ("topping" and "suckering") as rapidly as they appear, the upper few leaves on the stalk continue to increase in size for some time. Actually the upper third of the leaves on the stalk had a prolonged growth period in plants which were topped at the 21st leaf; and the 17th, 19th, and 21st leaves of these plants, as contrasted with corresponding leaves of untopped (normal) plants, show 32 per cent greater average area and 29 per cent greater average thickness.

2. This greater than usual growth of upper leaves of topped plants is due to a greater than usual increase in cell size. The palisade and upper and lower epidermal cells average 31 per cent larger. The fundamental tissue of the petiole does not increase in proportion to the tissues of the blade, its cells averaging only 23 per cent larger.

3. The only change in numbers of cells (in leaves 17, 19, and 21) due to topping occurs in the vascular tissue. Cambial activity results in an average of 47 per cent more lignified xylem elements in the petiolar bundle. Differentiation of secondary phloem in the petiolar bundles of these leaves is negligible, despite cambial activity.

4. The following correlations between structure and function in the upper leaves of topped plants seem worth noting: (a) There are fewer secondary phloem cells in the petiolar bundles than in corresponding leaves of untopped plants. This suggests that there is a partial cessation of phloem differentiation associated with the discontinuance of translocation of material out of the leaves to develop-

ing flowers and seeds. (b) The degree of development of the xylem and the amount of water loss are closely interdependent. This is evident from the fact that veinage increases in almost constant proportion to the enlarging leaf blade, and is accompanied by the development of considerable secondary xylem in the petiole.

Appreciation is expressed to Miss EDITH TOMKINS for her assistance in the determinations of cell sizes.

CONNECTICUT COLLEGE
NEW LONDON, CONNECTICUT

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EFFECT OF ULTRAVIOLET RADIATION ON GROWTH AND ON THE CALCIUM AND PHOSPHORUS CONTENTS OF PLANTS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 458

HARRIS M. BENEDICT

(WITH PLATE II AND ONE FIGURE)

Introduction

With the discovery that ultraviolet radiations between 2900 and 3100 Å. caused an increase in the calcium and phosphorus contents of the blood of animals, plant physiologists began investigations to determine whether or not these rays were of importance to plant growth. To date these investigations, which have been so thoroughly reviewed by POPP and BROWN (7) that it is not necessary to discuss the literature here, have not been able to show conclusively that these wave lengths have much effect on the dry weight or on the organic constituents of the plant. With one or two exceptions (BEESKOW 4, and FULLER 10), however, they have neglected the ash elements of the plant.

At the time this investigation was started, no paper had appeared on the effect of ultraviolet radiation between 2900 and 3100 Å. on the calcium and phosphorus contents of plants, and the reported results were so conflicting and open to question that it seemed advisable to investigate this subject further.

For any radiation to affect the object it strikes it must be absorbed by that object; therefore if certain wave lengths are transmitted by a leaf they cannot affect it. If the epidermis of a leaf transmits certain wave lengths which the rest of the leaf absorbs, however, the radiation absorbed will bring about some change in the interior of the leaf. It may be only a rise in temperature, but it may also bring about chemical changes such as in photosynthesis, or it may modify or decompose the constituents of the protoplasm such as enzymes, sugars, amino acids, etc. It is important therefore to study the absorption of the ultraviolet rays by the leaf and to observe any differences that

may occur. In investigations in which glass filters and quartz mercury arcs were used to furnish the different regions of the ultraviolet, the possibility was kept in mind that these glass filters might leak a small amount of the harmful rays shorter than 2900 Å., which would offset any beneficial effect derived from the region between 2900 and 3100 Å. On the other hand, in plants which were stimulated under these conditions it was possible that the upper epidermis might filter out these harmful rays, preventing them from reaching the photosynthetic cells directly beneath the epidermis.

From some preliminary experiments to determine the effects of this region of ultraviolet on plant growth, there were indications that after the plants were about four weeks old these radiations had no effect whatever. It seemed possible that this lack of influence might be explained on the grounds that with increasing age the epidermis became more opaque to these wave lengths, finally absorbing all of them, so that they could have no effect on the photosynthetic cells in the interior of the leaf.

As no conclusions as to the capacity of transmission of the leaf epidermis, nor as to the changes in such capacity with age, could be found from previous publications, spectrograms were made of the light transmitted by leaves and their upper epidermises, employing species previously used in ultraviolet experiments.

Methods

Three compartments 12 feet long, 3 feet deep, and 7 feet high were constructed. Each of these was protected from radiations from any other compartment. The only light other than the lights hung in the compartments came in through small windows in the north side of the room, and this extraneous light was kept from the compartments by black curtains hung across the front of them.

In each compartment were hung two 1000 watt tungsten lamps with reflectors and a Cooper-Hewitt Uviol mercury arc equipped with an aluminum reflector. The mercury arcs were suspended between the tungsten lamps and provided the blue and ultraviolet radiations, the tungsten lamps furnishing the longer wave lengths. No attempt was made to filter out the infra-red radiations present.

The ultraviolet radiations were controlled by the filters described

by WITHROW (9). The arc in compartment I was not filtered, but the intensity was reduced to that of the other compartments by the use of three layers of no. 600 Du Pont cellophane. The radiations in this compartment ranged from 2650 to 7200 Å. plus the infra-red rays. The mercury arc in compartment II was filtered with two layers of no. 600 cellophane soaked in a solution of sodium benzoate. This filtered out all the wave lengths shorter than 2900 Å. The mercury arc in compartment III was filtered with two layers of the same

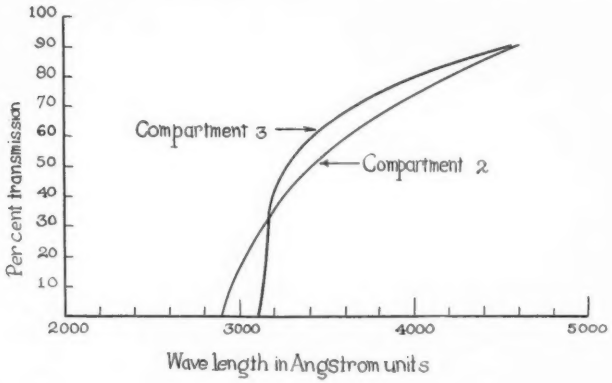


FIG. 1—Absorption spectra of filters

cellophane soaked in a solution of potassium acid phthalate. This filter absorbed all radiations shorter than 3130 Å.

The absorption curves of these filters are shown in figure 1. As can be seen from the curves, the energy transmitted by the two filters is practically identical, the areas under the two curves being approximately the same. Thus compartments II and III have the same intensity but different wave lengths of ultraviolet radiation. The intensity of the visible radiation in all three compartments was measured with a Macbeth illuminometer and found to be the same, with an intensity equal to 200 foot-candles. The amount of infra-red radiation is not known, but inasmuch as the cellophane filters have little absorption in the infra-red region, it seems safe to assume that the intensity in all the compartments was the same. Recent work by ARTHUR (2) seems to indicate that infra-red rays have little or no

effect on the dry weight of plants. These filters were changed every two weeks and showed no change in transmission in that time. The lights were hung at a distance of 40 inches from the tops of the pots placed in the compartments.

The temperature and humidity of the compartments were not controlled, but records show that the temperatures of the compartments never varied more than 2° C. from one another, with a mean around 25° C. The humidities of the compartments never varied more than 10 per cent, with an average humidity of 50 per cent.

The soil used was a rich loam. It was mixed thoroughly before using to insure equal soil conditions for all the pots in the three compartments. The soil was sterilized and placed in 8 inch pots, forty of which were placed in each compartment. While the plants were growing, the pots were supplied with an equal amount of water once a day.

The seeds were planted and the lights turned on immediately. The lights were in operation from 6:00 P.M. to 8:00 A.M. daily, during which period the curtains were left open in order to keep the temperature as low as possible.

Three series of plants were grown. The first series was planted November 22, 1931 and harvested one month later on December 22. This series will be referred to as series A. The second series was planted January 14, 1932 and harvested two months later, on March 14. This series will be referred to as series C. The third series was planted April 1, 1932 and harvested about six weeks later on May 19. This, which will be referred to as series B, was killed by escaping ammonia gas, but was harvested immediately; it is not felt that for these determinations the results were affected seriously. Each series can be divided into three sets. Set 1 consists of plants grown in compartment I and receiving ultraviolet radiations as short as 2650 Å.; set 2, of those plants grown in compartment II and receiving wave lengths as short as 2900 Å.; set 3, of those plants grown in compartment III and receiving wave lengths as short as 3100 Å. The upper limit of wave length all the sets received was 7200 Å. plus the infrared rays. The conditions of the three sets of any one series were as nearly alike as possible, with the exception of the wave length of ultraviolet present.

When ready for harvesting the plants were washed from the pots and the entire plants dried at 100° C. for one hour, then at 60° C. for 12 hours in a ventilated dryer. The plants were then weighed and dry weights recorded.

Calcium was determined by the method involving titration with 0.1 N potassium permanganate (3). Phosphorus was determined by the method outlined by ROE, IRISH, and BOYD (8) for blood phosphorus as modified by COCKEFAIR (5) for use in plant analyses.

The experiments with the penetration of ultraviolet were carried out as follows: A hydrogen arc operating at 4400 volts and one-half ampere was used as the source of radiations. The spectrograms were taken with a Hilger E 3 spectrograph.

The leaves were left on the plant while the spectrograms were being taken. They were placed in front of the slit, held firmly in a flat position, and exposed to the radiation for 20 minutes. This length of exposure was used after it had been found that it did not produce much injury to the leaf, but was of ample length to affect the plate.

In exposing the upper epidermis only, the leaf was placed with this epidermis down on a smooth surface and the lower epidermis and the mesophyll carefully scraped away, leaving only the upper epidermis. This was then examined with a hand lens to be sure that no holes were present. The epidermis was then exposed to the radiation in front of the spectrograph for 30 seconds. It is admitted that this is rather severe treatment, and the epidermis under these conditions may not be normal, but the procedure seems less likely to modify the optical properties than would stripping the epidermis from the leaf.

Results

The results of the determinations of dry weight, calcium, and phosphorus contents of the plants of series A, B, and C are shown in tables I, II, and III respectively. The dry weights are expressed in grams per plant, and the calcium and phosphorus contents as percentage of the dry weight.

In general there is not much difference between the dry weights of the plants of the different sets of series A. In series B and C the dry weights of the plants of set 2 are on the whole larger than those

of sets 1 and 3, with the exception of corn and sunflower in series C where the plants of set 1 have the greatest dry weights.

In all the plants of the three series the calcium contents of those in set 2 are higher than those in set 3, with the plants of set 1 usually

TABLE I

NUMBER OF PLANTS, DRY WEIGHT PER PLANT, CALCIUM AND PHOSPHORUS CONTENTS OF PLANTS OF SERIES A

PLANT	SET 1				SET 2				SET 3			
	NO. OF PLANTS	WEIGHT PER PLANT (GM.)	CALCIUM CONTENT (%)	PHOSPHORUS CONTENT (%)	NO. OF PLANTS	WEIGHT PER PLANT (GM.)	CALCIUM CONTENT (%)	PHOSPHORUS CONTENT (%)	NO. OF PLANTS	WEIGHT PER PLANT (GM.)	CALCIUM CONTENT (%)	PHOSPHORUS CONTENT (%)
Tomato	30	0.011	3.6	.....	31	0.007	2.6	.....	28	0.005	2.1	.....
Sunflower	.....	.....	.....	.....	40	0.137	3.3	0.62	40	0.205	2.9	0.67
Corn	30	0.173	1.5	0.76	28	0.141	2.0	0.70	23	0.172	1.1	0.63
Soy bean	17	0.161	1.8	0.73	49	0.150	2.4	0.62	43	0.158	1.6	0.67
Lupine	10	0.131	1.7	0.70	29	0.139	1.8	0.77	28	0.141	1.6	0.64

TABLE II

NUMBER OF PLANTS, DRY WEIGHT PER PLANT, CALCIUM AND PHOSPHORUS CONTENTS OF PLANTS OF SERIES B

PLANT	SET 1				SET 2				SET 3			
	NO. OF PLANTS	WEIGHT PER PLANT (GM.)	CALCIUM CONTENT (%)	PHOSPHORUS CONTENT (%)	NO. OF PLANTS	WEIGHT PER PLANT (GM.)	CALCIUM CONTENT (%)	PHOSPHORUS CONTENT (%)	NO. OF PLANTS	WEIGHT PER PLANT (GM.)	CALCIUM CONTENT (%)	PHOSPHORUS CONTENT (%)
Corn	19	0.516	1.9	0.26	16	0.621	1.5	0.68	22	0.515	1.3	0.49
Tomato	30	0.011	3.0	0.35	23	0.185	3.5	0.30	23	0.150	3.2	0.58
Nasturtium	59	0.167	2.4	0.45	50	0.192	2.6	0.47	59	0.166	1.9	0.56
Soy bean	16	0.260	2.0	0.35	28	0.493	2.0	0.54	23	0.460	1.8	0.43
Cucumber	31	0.100	4.4	0.34	21	0.381	4.2	0.54	21	0.258	2.7	0.38

having a calcium percentage intermediate between those of sets 2 and 3.

The percentage of phosphorus is about the same in all three sets of series A. The plants of sets 2 and 3 of series B are about equal in the phosphorus content, which is somewhat higher than that of set 1; while in series C the plants of set 3 have the greatest phosphorus content.

The results of the studies of the penetration of ultraviolet radiations through the epidermal tissues and the entire leaves are shown in tables IV and V. In all cases the transmission of radiation was

TABLE III

NUMBER OF PLANTS, DRY WEIGHT PER PLANT, CALCIUM AND PHOSPHORUS CONTENTS OF PLANTS OF SERIES C

PLANT	SET 1				SET 2				SET 3			
	NO. OF PLANTS	WEIGHT PER PLANT (GM.)	CALCIUM CONTENT (%)	PHOSPHORUS CONTENT (%)	NO. OF PLANTS	WEIGHT PER PLANT (GM.)	CALCIUM CONTENT (%)	PHOSPHORUS CONTENT (%)	NO. OF PLANTS	WEIGHT PER PLANT (GM.)	CALCIUM CONTENT (%)	PHOSPHORUS CONTENT (%)
Sunflower	48	0.230	3.5	0.71	50	0.199	4.0	0.68	32	0.168	3.6	0.68
Cucumber	20	0.279	3.9	0.45	18	0.441	5.0	0.52	22	0.226	3.4	0.66
Tomato	21	0.115	3.6	0.65	22	0.263	4.1	0.60	28	0.165	3.1	0.68
Corn	31	0.875	1.5	0.39	32	0.735	1.5	0.51	31	0.859	1.1	0.58
Soy bean	22	0.364	2.0	0.49	20	0.504	2.2	0.33	19	0.455	0.8	0.54
Nasturtium	48	0.161	3.1	0.76	38	0.228	2.6	0.54	50	0.137	1.9	0.71

TABLE IV

LOWER LIMITS OF TRANSMISSION (Å.) OF LEAVES AND EPIDERMISES

PLANT	LEAF	EPIDERMIS
Corn	3750	2350
Morning glory	3420	2250
Cucumber	3250	2400
Tomato	3850	2240
Wax bean	3850	2250
Bryophyllum	.....	2500
Radish	3500	2300
Coleus	3800	2600
Sunflower	3550	2260
Soy bean	3500	2500
Sudan grass	3450	2260

continuous down to a certain wave length, with no radiation shorter than that wave length transmitted. Table IV shows in the second column the shortest wave length transmitted by the entire leaf and in the third column the shortest wave length transmitted by the upper epidermises of the leaves.

Table V shows the transmission of leaves and their upper epider-

mises at the ages of two, three, and five weeks. The results show a slight decrease in the transmission of both the leaf and the upper epidermis with increase in age. In no case does the upper epidermis filter out all the wave lengths shorter than 2900 Å. Plate II shows a typical spectrogram of the transmission of various leaves and their epidermises.

TABLE V

TRANSMISSION (A.) OF LEAF AND EPIDERMIS OF VARIOUS PLANTS AT DIFFERENT AGES

PLANT	LEAF			EPIDERMIS		
	AGE IN WEEKS			AGE IN WEEKS		
	2	3	5	2	3	5
Wax bean	3250	3450	3500	2300	.....	2310
Corn	3520	3740	4050	2250	2400	2380
Lupine	4800	.....	4800	2270	.....	2300
Radish	3330	3340	3800	2240	2240	2390
Sunflower	2900	.....	3550	2230	.....	2290
Cucumber	3150	.....	3250	2360	.....	2460
Tomato	3800	3850	3950	2220	2240	2240

Discussion

The chief criticism of the conditions under which the plants in these experiments were grown was the low light intensity. For good growth the intensity in the compartments should have been around 1000 foot-candles instead of 200. Since the intensity in all the compartments was the same, however, the results can be considered as indications of the effect of the wave lengths between 2900 and 3100 Å. on the growth of the plants studied; but because of the low light intensity they can be considered as indications only.

ARTHUR (1) has recently stated that the potassium acid phthalate filter transmits certain wave lengths shorter than 3100 Å. This is true if the filter is not used with a Corex D or Uviol glass backing. If this filter is backed with one of these glasses, however, as it was in these experiments, there is no transmission of wave lengths shorter than 3100 Å.

The results of the dry weight determinations on the plants of series A indicate that any effect the ultraviolet regions between 2900

and 3100 Å. and between 2650 and 2900 Å. might have on the growth of the plants does not become apparent until after the plants are a month old, since the plants of the different sets of this series showed little differences between their dry weights. The plants of sets 1 of series B and C, which were six weeks and two months old respectively, showed the harmful effects of the rays between 2650 and 2900 Å. in their decreased dry weight and in the burned appearance of the leaves which were exposed to these radiations. The plants of these two series also showed the possible benefit of the wave lengths between 2900 and 3100 Å. in the increased dry weight of the plants of set 2 which were exposed to these radiations as compared with that of the plants of set 3 which did not receive these wave lengths. This increase in dry weight was most marked in the case of tomatoes and cucumbers, being as much as 90 per cent in the cucumber plants of series C. In both series B and C there was enough difference between the plants of the different sets to tell them apart readily. The plants of set 2 were the largest, with the exception of corn in series C; those of set 3 were somewhat smaller; those of set 1 were usually of a stunted appearance with their leaves inrolled and looking burnt.

From the results obtained under the conditions of these experiments, there are indications that in general wave lengths between 2900 and 3100 Å. stimulate plant growth, and that wave lengths shorter than 2900 Å. are harmful to plant growth.

The calcium content of the plants exposed to the wave lengths between 2900 and 3100 Å. was increased, both in percentage and in total amount per plant. All the plants of sets 1 and 2 in all three series showed this increase in percentage of calcium. The plants of set 2 usually had a slightly higher percentage than those of set 1. These results seem to indicate that the wave lengths between 2900 and 3100 Å. are most effective in bringing about this increase in calcium content, and that the wave lengths shorter than 2900 Å. exert very little influence, possibly even interfering with the intake of this element, since the percentage of calcium in the plants of set 1 was generally not so great as in the plants of set 2.

This increase in calcium started with the early development of the plants. The plants of series A were only a month old and showed no significant differences in their dry weights, yet the increase in the

percentage of calcium in the plants of sets 1 and 2 was already apparent. There are no indications in the results that the percentage of calcium increases or decreases with age. For a given series the soil of the sets was identical (that is, all the plants of one series were grown in the same soil), but the soil used in series B was not the same as the soil used in series A, and the soil used in series C was not the same as was used in series B. This difference between the soils of the different series may account for the differences noted between the plants of the three series.

The results of the calcium determinations seem to indicate that wave lengths between 2900 and 3100 Å. bring about an increase in the calcium content of the plants, and that wave lengths shorter than 2900 Å. have little effect on the intake of this element.

The results of the phosphorus determinations are peculiar. The plants of set 1 of series A show an increase in phosphorus over the plants of set 3, but in the other two series this set shows on the whole a decrease in percentage from that of set 3. The plants of set 2 show an increase in phosphorus in series A and B, but a rather decided decrease in series C, sunflower and nasturtium being exceptions. These results are so variable, however, that it does not seem safe to make deductions as to the effect of these regions of wave lengths on the phosphorus content of the plants studied.

The results of the study of the transmission of the leaf and upper epidermis with increase in age do show a possible increased absorption of radiations as the age increases, both in the leaf and in the epidermis. In most cases the variations do not exceed the limits of variation between leaves of the same age, with the possible exception of corn. Likewise the increase in absorption of ultraviolet rays by the epidermis with increase in age does not become great enough to filter out all the radiations shorter than 3100 Å.

The results of the penetration studies show that in no case did any wave length shorter than 2900 Å. penetrate through the leaf, and in no case did the upper epidermis filter out all the wave lengths shorter than 2900 Å. ELTINGE (**6**) reported that in the case of tomato, cucumber, radish, and *Coleus* she obtained stimulation by adding the wave lengths between 2900 and 3100 Å., but no stimulation in the case of morning glory, wax bean, and sunflower. The results presented here show that the epidermis could not have acted

as a protection against short wave lengths leaking through faulty filters in the case of the stimulated plants, and at the same time have failed to act as a protection to the plants that were not stimulated, since the transmission of the epidermis of all the plants was about the same.

The evidence presented by these tests indicates that any results obtained with ultraviolet radiation experiments must always be interpreted on the basis of the penetration of rays shorter than 2900 Å. through the upper epidermis of the leaf, and the absorption of these rays by the underlying tissues if they are present in the radiations.

It will be noted that some leaves absorb more ultraviolet radiation than others. This is in general related to the thickness of the leaves. The thick leaf of *Bryophyllum* transmitted no radiations at all; lupine with a relatively thick leaf absorbed all the ultraviolet, and wax bean with a leaf thinner than the lupine leaf absorbed most of the ultraviolet radiations.

Summary

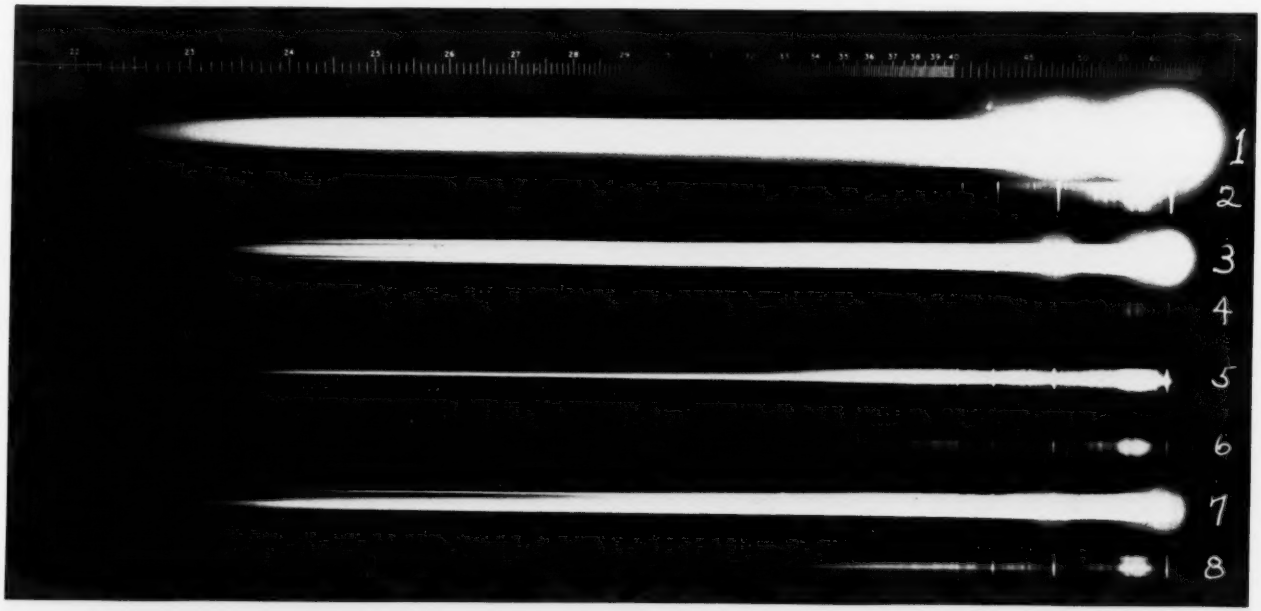
1. Three series of plants were grown and each series was divided into three sets. All the conditions of the three sets of one series were identical with the exception of the range of wave length of ultraviolet radiation received.

2. The amount of growth of the plants was determined by measuring the dry weight. The calcium and phosphorus intakes were also determined for these plants grown in the three different ranges of wave length.

3. The region of ultraviolet between 2900 and 3100 Å. apparently caused, under the conditions of these experiments, an increase in the dry weight of all the plants grown with the exception of corn. Plants receiving no wave lengths shorter than 3100 Å. were considered as controls.

4. This same region of ultraviolet (2900–3100 Å.) apparently caused an increase in percentage of calcium in all of the plants grown. No conclusions could be drawn from the results as to the effect of these wave lengths on the phosphorus content.

5. The presence of wave lengths shorter than 2900 Å. caused a decreased dry weight of the plants but had little effect on the calcium content.



BENEDICT on ULTRAVIOLET RADIATION

Spectrograms of various leaves and their epidermises. Reading from top to bottom: 1, hydrogen arc; 2, tomato leaf; 3, tomato epidermis; 4, lupine leaf; 5, lupine epidermis; 6, radish leaf; 7, radish epidermis; 8, soy bean epidermis. Some faint lines showing the lowest limits of transmission are lost in reproduction.

6. Transmission studies with spectrograms indicate that the upper epidermis cannot filter out harmful wave lengths of ultraviolet, and that its transmission of ultraviolet radiations does not decrease with age.

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CENTRAL GREAT PLAINS HORTICULTURAL FIELD STATION
CHEYENNE, WYOMING

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STUDIES ON PETUNIA. VI

THE ORIGIN AND DISTRIBUTION OF COLOR IN THE ANTHER AND IN THE POLLEN OF PETUNIA[1]

MARGARET C. FERGUSON AND BARBARA HUNT

(WITH PLATE III)

A recent paper (FERGUSON **6**) deals with the inheritance of pollen color in diploid petunias. The present paper is concerned especially with the origin, the distribution, and incidentally with the nature, of the pigments in the anther and in the pollen of *Petunia*, including both diploid and polyploid strains.

Here and there in the literature dealing with pigmentation in plants occur scattered references to colored anthers and to color in the pollen, but we have not been able to find reports of any studies based primarily on the origin of color in the anther and in the pollen. However, various writers in the early part of this century were evidently in accord with the view expressed by EAST (**4**) that "it is well known that pollen color is a tapetal deposit." We are aware of no definite researches on the subject which would give rise to such a generally accepted idea. It may be that the belief has grown out of VAN TIEGHEM'S (**17**) discussions. He described the growth of the tapetal cells which he said ordinarily take on a yellowish color, and form a complete sheath of yellow cells enveloping the pollen mother cells. He noted that later these cells break down and nourish the young pollen grains, the exine of each grain being yellow in color throughout its entire thickness.

In 1923 MÖBIUS (**13**) studied the color of anthers and of pollen in 120 species of plants. The list does not include *Petunia*. Eighty per cent of the species investigated had yellow pollen. He states that in about 20 per cent of these the color is due to a yellow oil which is produced by the tapetum and adheres to the pollen grains. He cites KERNER (**11**) as having found among 520 species, 400 with a

[1] Read before the Botanical Society of America at the Boston meetings, December, 1933.

very thin layer of fatty oil on the outer surface. We do not find, either in the original treatise or in OLIVER'S (12) translation, any evidence that KERNER associated this fatty oil, which he says is usually yellow, sometimes colorless, with pollen color or considered it a factor in the determination of pollen color. There is undoubtedly a very close connection between the yellow pigment of the tapetum and that of the pollen grains; but whether the coloring matter of the latter is secreted by the former, or is synthesized independently in the pollen has not been definitely determined by earlier investigators.

Methods and observations

GENERAL DISCUSSION

When the anthers are fully mature, that is, just prior to their dehiscence, their color may be yellow, varying in different strains from very light to very dark yellow, light gray-blue, light gray-green, bluish purple, or reddish purple. Very rarely we find strains in which the light yellow anthers may contain no pigment. Their apparent color is due to the fact that their walls, composed of white semitransparent cells, inclose yellow pollen. In some strains the anthers are not uniformly colored but present a mosaic. In such cases the background is invariably some shade of yellow on which are streaks or splashes of reddish or bluish purple. The pollen of petunia varies greatly in color. The anther and pollen may have practically the same color, although this very rarely occurs. As a rule they are slightly or markedly different. We have already referred to white anthers carrying yellow pollen. Our large-flowered, deep purple strain, population eight, has, in RIDGWAY (16) terminology, Baryta Yellow anthers and Turtle Green pollen. Our results as to variation of color in these organs are in substantial agreement with MÖBIUS' findings, except that he notes a much smaller range of color in the pollen than do we. This difference is doubtless due to the fact that he worked with species only and our observations are based not only on the species but also on their hybrids.

Both red and blue anthocyanin may occur in the anthers and in the pollen of *Petunia*. Neither color has been found to be present in these organs without the other. Ordinarily they are associated in the same cell, giving tints of purple; but in some instances, as in the

anthers of *P. violacea*, the red and the purple may at times be found in adjacent cells, making a very beautiful mosaic as viewed in section under the microscope. The special "color bodies," to be described later, which are associated with the pollen of polyploid petunias, may contain a mixture of the two anthocyanins, or some may carry blue and others red anthocyanin. Since we so rarely, if ever, find clear blue or clear red in *Petunia*, it would probably more nearly express the actual conditions existing in the plant to say that red anthocyanin may be greatly in excess of the blue in some anther cells and in certain of the "color bodies," and blue in excess of the red in others. The conclusion regarding the presence of two anthocyanins is not based on microscopic observation alone. In comparing the anthers and the pollen, which evidently contain anthocyanin, with the RIDGWAY (16) colors, they are invariably found to correspond to colors which are made up of a mixture of red and of blue pigments. Also the tests for anthocyanin as outlined by BUXTON and DARBISHIRE (3) give very clear evidence of the presence in anther and in pollen of both red and blue anthocyanin.

As reported by ONSLOW (14), BOYLE (2) was the first to use the now classical experiment of fuming white flowers with ammonia. As a result of the fuming his flowers became greenish yellow. FILHOL (8) gave the name xanthogène to the substance responsible for this yellow color and later, 1860, he noted its similarity to luteolin; but not until 1905 was its identity with flavone pigments established by BIDGOOD (1). When the anthers or the pollen of different species or of cultivated strains of petunias are subjected to ammonia fumes, the results give positive evidence of the presence of at least two flavones, a lighter and a darker yellow, in both of these organs.

ANTHER

Since certain of the pigments found in the anthers of *Petunia* are soluble in water, alcohol, and various other liquids, it is not feasible to study their origin and distribution in fixed material. Our study is perforce based on free-hand sections. But with the freshly cut material we also encountered difficulties which could not have been anticipated. When pigment, other than green or yellow, is first observed in the growing anthers, it is invariably a beautiful magenta

red. Because of the ready solubility of this pigment, we were unable to find any altogether satisfactory medium in which to study the freshly cut sections. Rather thin sections of anthers in various stages of development were made from buds of flowers which, when fully open, bear purple anthers. It was found that when these are mounted either in water or in alcohol the magenta pigment disappears almost instantly. Sections were therefore mounted in various other media and immediately sealed with heavy balsam or in a few instances with vaseline.

This pigment is evidently soluble in both cold and hot chloroform, disappearing from the sections as viewed under the microscope in from 6 to 8 minutes. Of the various media used, benzine proved to be by far the best for studying the origin and the distribution of this red anthocyanin, if such it be, with benzene, xylol, and nujol next in order. But in all cases it was essential that the observations be made as promptly as possible after the mounts were prepared, as the magenta color quickly disappears. Whether it passes into solution or is changed chemically we are unable to say. In xylol, nujol, and benzene the bright pigment is lost in from 12 to 15 minutes; but in benzine from 20 to 30 minutes may elapse before any change in the amount or position of this pigment is detected. As the magenta color passes out, in the various media, a bluish pigment, more abundant than the magenta and sometimes spreading over the entire section, appears. As this change takes place, the identity of the cells which earlier carried the magenta red pigment is lost: and, in most of the solvents used, the blue coloration also disappears within an hour. Doubtless the red pigment found in the cells of the growing anthers of *Petunia*, although present in solution in the cell sap, is not an anthocyanin. It combines the solubilities of anthocyanins, carotenoids, and certain flavones. It is possible that it may belong with the red hydrocarbon pigments, which are not carotenoids, mentioned by PALMER (**15**). We have made no attempt to determine the chemical nature of this magenta pigment; to do so would take us into a field entirely apart from the purpose of this paper.

Just prior to the maturity of the anthers a change occurs both in the color and in the constitution of the bright pigment. The violet or reddish or bluish purple pigment, which during the final steps in

the maturation of the anther replaces in part the red pigment, is doubtless a true anthocyanin. It is soluble in water and in alcohol and insoluble in chloroform, benzene, and similar substances.

The young anthers show no color other than the green which is due to the presence of chloroplastids. But coincident with the formation of the microspore mother cells and the differentiation of the tapetum, a magenta red cell sap appears at four points in the anther as seen in cross-section. Five to seven pigmented cells are grouped at the center of each of the dome-shaped masses of parenchyma tissue which are capped by the four microsporangia (pl. III, fig. 1). When the tapetum has become multinucleated and microsporogenesis is completed, the number of cells containing the magenta red pigment has considerably increased, but this color does not occur outside the cones of sterile tissue (fig. 2). During the period of transition from microspores to pollen grains, the tapetum largely disappears, the endothecium becomes prominent, and the amount of magenta pigment is greatly increased. It has extended much more deeply into the cells of the sterile plates separating the two sporangia on either side of the anther, and has appeared in a very limited number of cells in the inner portion of the anther wall (fig. 3).

Following the maturation of the pollen grains and of the endothecium, the cells connecting the sterile plates and the wall of the anther separate in the line of dehiscence and the two sporangia on either side become confluent. As these changes are taking place, the pigmented cells become very abundant and are scattered throughout the broad plates of sterile tissue (fig. 4). During the entire development of the anther, from the time when pigment other than green or yellow first appears, up to the structural conditions represented in figure 4, no colored cell sap other than the magenta red is present in the anthers of any strain of *Petunia* which we have studied. Just prior to dehiscence, however, a marked change takes place in the pigmentation of the anther, and a violet, a red-purple, or a blue-purple anthocyanin becomes abundant. If the sections are cut at just the right moment, one can see, at points lateral to the connective and on both the anterior and posterior sides of the anther, a violet or a purple anthocyanin extending from the cells with the magenta cell sap at opposite ends of the sterile plates out into the wall of the anther.

The points of origin of the anthocyanin and its line of progression outward are indicated by the arrows in figure 4. In other anthers, the two lines of progressive pigmentation on either side have almost met; and in still others the entire anther wall, with the exception in some cases of the epidermis, is colored with anthocyanin. When the anther is about to dehisce, some of the magenta color ordinarily remains, but in large measure it has lost its vivid color and is replaced by the duller colored anthocyanin. Whether it is actually connected with the origin of the anthocyanin or whether the two simply develop in close proximity we cannot say. The fact that the magenta pigment is at least partially lost, being replaced by the anthocyanin, suggests that the latter originates at the expense of the former.

MÖBIUS (13) found anthocyanin present in the endothecium only in rare instances. When the mature anthers of *Petunia* are uniformly colored over their entire surface, the cells of the endothecium are even more deeply colored than are the other cells of the anther wall.

While as a rule the red and the blue anthocyanins are mixed in the same cell, giving a reddish or bluish purple pigment, we occasionally find, as already mentioned, an exception to this in the anthers of *Petunia violacea*. In this species the anthers may carry a reddish purple anthocyanin, uniform in color throughout all the cells of the wall; but in some instances many cells of the inner layer of the mature anther wall are seen to carry a red pigment, lightly tinged with blue.

In those cases in which the anthocyanin is confined to certain regions of the anther, we have not been able to trace any connection between the color in the cells of the outer wall of the mature anther and the magenta pigment present in the cells of the sterile plate. As the anthers become biloculated, just prior to dehiscence, a bright, reddish purple color appears in scattered cells of the epidermis and here and there in the cells of the inner portion of the wall. We have never found anthocyanin in the cells of the endothecium when the pigment is so distributed in the anther as to form a mosaic (fig. 4).

We have noted earlier that all the cells of the very young anthers contain chlorophyll. Shortly before the origin of the microspores, much of the chlorophyll green is lost and there appears a light yellow pigment rather generally distributed throughout the tissues of the anther. This is a common phenomenon in connection with the loss

of chlorophyll. This yellow pigment gradually becomes more or less condensed, depending on the particular strain of *Petunia*, in the cells of the inner layers of the wall, more especially in the disintegrating cells lining the pollen chambers, and in the pollen grains.

The yellow pigments of the anthers are present in the form of soluble flavones, of chromoplastids, and of an insoluble pigment which may or may not be an insoluble form of a flavone. A portion of the yellow color is soluble in water, and another portion in alcohol; but there remains a yellow pigment which has not passed into solution in any of the solvents with which we have treated it. We shall consider in more detail the solubility of this yellow pigment in our discussion of the pollen grains.

POLLEN

In all our cultures of *Petunia*, the mature pollen grains have color. The great variability in color which they present was mentioned earlier. We (**6**) have recorded to date 38 shades or tints in our various populations breeding true for color, and in their hybrids. These colors fall into the four main groups, blue, yellow, green, and gray. As described in previous papers (**5, 6, 7**), the typical pollen grains of *Petunia* are ellipsoidal (figs. 5, 6). They are very constant in shape and color in the species and in the true-breeding cultivated strains. In the species and in most other diploid strains, the color of the pollen mass is due to the color of the individual grains. But in polyploid strains, and in diploids with a high degree of heterozygosity, the grains are very irregular in shape (fig. 7 *a*) and, in addition to the color given by the individual grains, there are present very deeply colored structures which we have called color bodies (fig. 7 *b*). These doubtless represent pollen grains which have ceased to grow when only partially developed. As a rule their walls have become excessively thickened and they have acquired, doubtless as the result of an increased acidity in their contents, a very dense solution of anthocyanin. When anthocyanin is not present in the pollen grains, these bodies are bright yellow. The small collapsed pollen grains, evidently dead cells containing some pigment (fig. 8), are not included in our conception of the color bodies.

We agree with MÖBIUS that it is not easy to decide, by use of the

microscope, in which part of the pollen grain the pigment resides. He found only three species in which there was any color in the contents. In a few cases he ascribes, as stated earlier, the color of the pollen to color granules or to a fatty oil adhering to the outer surface of the grains; but in the great majority of the species investigated he found color in the exine only. GERTZ (10) studied the distribution of pigment in over 1500 species and concluded that only very rarely can anthocyanin be said to be connected with the cell membrane. After repeatedly studying pollen grains, in both end and lateral views, after crushing them on the slide, and in certain colorless media which cause their contents to be partially extruded, we have no hesitancy in stating that both exine and contents of the pollen grains of *Petunia* carry pigment. As a rule the exine is more deeply stained with pigment than are the contents. We find no evidence that color in the pollen grains is derived from any special portion of the anther. It seems clear that the conditions conducive to the formation of anthocyanin, or of yellow pigment, in the anther also induce the production of these pigments in the pollen grains. There is no evidence whatsoever that the pollen grains are receptacles of pigments organized and passed on to them by other tissues of the plant.

After extracting the anthocyanin from the anthers and the pollen grains of *Petunia*, they are invariably yellow. This is most strikingly demonstrated in the pollen grains, and leads us to conclude that in the anthers and in the pollen of *Petunia* there is, as it were, a foundational yellow pigment. If yellow anthers and yellow pollen are boiled in water for 15 minutes, the liquid becomes distinctly yellow. A microscopic examination shows that anther and pollen are still yellow. After drawing off the water, adding 95 per cent alcohol and boiling for half an hour, the liquid is again yellow; but anther and pollen are also yellow, the pollen a deeper yellow than the anther. Both the walls of the grains and the crushed-out contents still contain a yellow pigment. Exactly the same results are obtained when 50 per cent hydrochloric acid is used. Sealed mounts were made of yellow anthers and yellow pollen after having boiled them for half an hour each in water, in alcohol, and in hydrochloric acid. After three weeks, the grains and anthers in all three media were still yellow. When these organs are boiled for 10 minutes in ether, in benzene and

similar substances, or in weak sulphuric acid, the liquid is a brighter yellow than after boiling in alcohol, the anther wall is a very light yellow, and both the exine and the partially extruded contents of the pollen grains are distinctly yellow. It is such results as these that have led us to conclude that the yellow pigment in both anthers and pollen of *Petunia* consists not only of carotenoids and soluble flavones, but that a yellow pigment in an insoluble form is also present; and that this insoluble yellow pigment is found in all the pollen grains which we have studied whether or not anthocyanin be present. This insoluble yellow pigment is doubtless identical with, or closely related to, the yellow pigment which has been preserved in the color of spores and of pollen grains during all the eons of time since the coal beds were laid down.

Summary

1. Throughout this paper, all conditions described or conclusions reached are meant to apply only to those species or strains of *Petunia* which we are growing in our cultures, even though such a limiting phrase may be omitted. At the same time we feel that our observations have probably covered a sufficiently wide range of forms to be of rather general application within the genus.

2. All cells of the very young anthers, except those of the connective, carry chlorophyll.

3. As the chloroplasts are lost, the yellow pigment of the older anthers and of the pollen is initiated.

4. No matter what the color of the mature anther and pollen may be, yellow pigment is always present in them although frequently masked by anthocyanin.

5. The yellow pigment falls into three groups as to solubility, a portion being soluble in water, a second portion soluble in alcohol, and a third portion insoluble in all liquids to which it was subjected.

6. The presence of two flavones is demonstrated.

7. Color, other than green or yellow, first appears in the sterile tissue at the inner side of each of the four microsporangia. It is in solution in the cell sap.

8. This pigment is always a magenta red and is soluble in all the media in which it was mounted. It is evidently not an anthocyanin.

9. True anthocyanin appears just prior to the dehiscence of the

anther. It is first seen in connection with the cells, containing the magenta cell sap, at either end of the sterile plate and from thence progresses outward into the wall.

10. The magenta pigment is evidently closely associated with the origin of the anthocyanin.

11. Both a red and a blue anthocyanin is present.

12. In uniformly colored anthers the anthocyanin may be present in all the cells of the anther with the exception of the connective.

13. With a mosaic distribution of the pigment in the anther, no anthocyanin has been observed in the endothecium.

14. Color is present in both the walls and the contents of the pollen grains.

15. In markedly heterozygous diploids and in polyploids, the color of the pollen is further strengthened by the presence of special color bodies.

16. All the evidence points toward a common origin of pigment in the anthers and in the pollen.

WELLESLEY COLLEGE
WELLESLEY, MASSACHUSETTS

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EXPLANATION OF PLATE III

Color in *Petunia*

All figures have been reduced $2\frac{1}{4}$ times in reproduction. The magnification of each drawing, as reproduced, is given. The cells containing the magenta pigment or the true anthocyanins are blackened.

FIG. 1.—Cross-section of young anther showing points of origin of magenta cell sap. Tapetum just organized. Microspore mother cells present. ×169.

FIG. 2.—Cross-section of one half of a slightly older anther. Tapetum fully organized. Microspore mother cells in early stages of meiosis. Cells with the bright pigment increased in number. ×111.

FIG. 3.—Cross-section of one half of a still older anther. Only remnants of tapetum remain. Endothecium fully established; microspores freed from mother cells and mature; pigment much more abundant and more widely distributed. ×78.

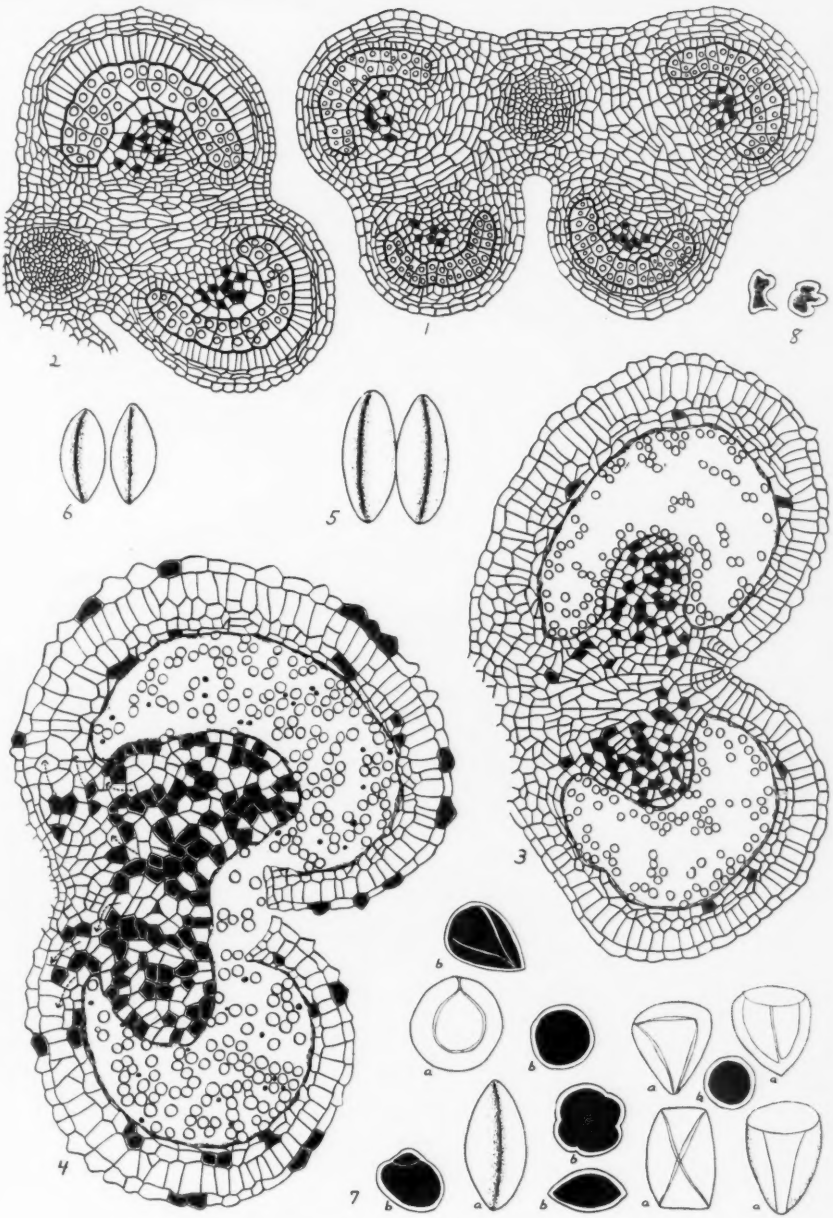
FIG. 4.—Cross-section of one half of an anther just prior to liberation of pollen. This illustrates an anther which has a mosaic color pattern when mature; but the arrows indicate place of origin and course of the anthocyanin when anthers are colored throughout at maturity. Pollen chambers have been formed; pollen is mature. ×78.

FIG. 5.—Typical pollen grains of *Petunia* as seen in *P. axillaris*. Both wall and contents are yellow in color. ×320.

FIG. 6.—Gray-blue pollen grains of *P. violacea*. Both wall and contents contain anthocyanin. ×320.

FIG. 7.—Characteristic pollen of a polyploid: fig. 7 *a*, some of the various shapes of the pollen grains which have pigment in both wall and contents; fig. 7 *b*, a few of the thick walled, densely pigmented color bodies. ×320.

FIG. 8.—Small, collapsed, dead pollen grains which are rather densely pigmented but are not considered as special color bodies. ×320.



FERGUSON & HUNT on PETUNIA

STRUCTURE OF MEIOTIC CHROMOSOMES IN MICROSPOROGENESIS OF TRADESCANTIA

KARL SAX AND L. M. HUMPHREY

(WITH PLATE IV)

Introduction

The coiled structure of meiotic chromosomes has been described in a considerable number of plants, including representatives of both gymnosperms and angiosperms. Although the origin and nature of the coiled structures have been considered by several investigators, there is considerable difference of opinion regarding their interpretation.

KUWADA (4), working with *Tradescantia*, interprets the coiled chromonema as a single coil containing the two chromatids in close association. The two chromatids are assumed to coil in such a manner that for each turn of the spiral there is a twist of the two chromatids about each other in the reverse direction. Such an arrangement of the chromatids would permit their separation without entangling. Such a coiled chromonema could be produced by the contraction of the chromosome pellicle, forcing the two closely associated chromatids into a single coil without rotating or twisting of the chromatids about each other. KUWADA's interpretation has been accepted by BRIDGES (ALEXANDER 1) and by SAX (9).

DARLINGTON (3), basing his argument on theoretical grounds, denies the existence of a single coiled chromonema, and states that "what appears to be a single spiral when considered as a perspective view is undoubtedly two separate spirals when considered as an optical section. . . . . The chromosome sometimes seems to be of a zigzag structure, and when apparently a spiral, the spiral may appear to reverse its coiling. This is no doubt due to one of the spirals being taken as a continuation of the other. Each *chromatid* is therefore one spiral, and there is no reason to doubt that this spiral is single and turns in one direction" (pp. 33). DARLINGTON also assumes that the coiling of the chromatids is controlled by the spindle

attachment, and that the direction of the spiral should always reverse at the fiber attachment (pp. 290). CATCHESIDE (**2**) has accepted this explanation of coiling of chromatids and suggests that such coiling "could be brought about by the revolution of the spindle-fibre attachment constriction about the long axis of the chromosome, the free ends of the chromosome being relatively stationary."

NEBEL (**7**) has analyzed in considerable detail the direction of coiling of both chromonemata and chromatids in the meiotic chromosomes of *Tradescantia*, and finds that the direction of coiling is more or less at random in the homologous chromonemata on either side of a terminal chiasma, or in the chromatids of the two arms of a single chromosome.

Chromosome coiling in Tradescantia

The present analysis of coiled chromonemata and chromatids was based on material from a *Tradescantia* species related to *T. reflexa* Raf. The species has not yet been described. This species was selected from the many available in Dr. EDGAR ANDERSON'S collection, because the anthers are relatively large at the time of meiosis. It is a diploid form with six pairs of chromosomes, and most plants carry one or more pairs of chromosome fragments. A few preparations were also obtained from an F_1 triploid produced by ANDERSON.

Previous investigators have found that various pretreatment methods bring out greater details of chromosome structure. The essential nature of the pretreatment methods commonly used seems to be one of slight dehydration before fixing the material. We have used natural desiccation and immersion in weak alcohol or sugar solutions. The method which gave most consistent results was immersion, after smearing, for 10 seconds in 20 per cent ethyl alcohol (50 cc.) made slightly alkaline by adding a few drops of ammonia water.

After the pretreatment in alcohol, the slide containing the smeared microspore mother cells was covered with fixing solution. Most of the common osmic fixatives gave good results, but a solution containing 2 per cent osmic acid and 1 per cent chromic acid was used most frequently. The preparations were fixed from 3 to 10 minutes and,

after rinsing with 30 per cent alcohol, were placed in 40 or 50 per cent alcohol for half an hour or longer. They were then stained with crystal violet according to Newton's schedule.

The chromosomes of the diploid *Tradescantia* are somewhat heterobrachial with submedian fiber constrictions. At the time of the first meiotic metaphase, both ring and rod bivalents are found. There are often one or two rod bivalents in the metaphase figures. The analysis of coiling of chromonema and chromatids at meiotic metaphase has been confined largely to these rod bivalents, because they can be studied more accurately.

At early metaphase it is perfectly clear that each homologue contains a single coiled chromonema (figs. 1, 2, 5). In most cases it is also clear that the chromonema contains the two chromatids coiled together in close association. The dual nature of the chromonema is shown by the parallel course of the chromatids across the entire width of the coil in many cases. This association is difficult to photograph, but it can be seen in the upper homologue shown in figure 6. In the clearer preparations the chromatids of the coiled chromonema occasionally appear to overlap and form a cross in the upper or lower level of the coil. The single coiled chromonema may persist until early anaphase (figs. 6, 7), although the separation of the coiled chromatids of each homologue is usually complete at this time.

At early metaphase, when the chromatids are associated in a coiled chromonema, the separation of chromatids can be observed at the terminal or subterminal chiasmata (figs. 2, 3, 5). During metaphase the coiled chromatids of each chromonema gradually separate until each homologue consists of two separate coiled chromatids. Various stages in the separation of coiled chromatids are shown in figures 4, 8, and 9, representing bivalents of the diploid form, and in figure 14, picturing two trivalents from the triploid hybrid. In most cases the two chromatids of each homologue are identical in regard to number of coils, direction of coiling, pitch of coils, and any minor irregularity which may be present.

As the chromosomes pass to the poles, each homologue shows clearly two coiled chromatids. In the earlier stages the coiled chromatids are parallel (figs. 10, 11, 12), but at late anaphase they separate, except at the spindle fiber, and form cross-shaped figures (fig.

13). During interphase the chromosomes elongate and tend to straighten out.

At the second meiotic metaphase the two chromatids of each chromosome are rod-shaped and appear much as they do in the usual somatic chromosomes. The chromosomes at this stage are about twice as long as they were at the first meiotic division. At this stage, and especially at early anaphase, it is clear that each chromatid has an internal coiled structure (fig. 15). There are about 20–25 secondary coils in each chromatid, as compared with four of five primary coils found at the meiotic division. These coils appear to be single, and careful study has shown no evidence of split *chromatids* at any stage of meiosis.

We have been unable to determine the direction of coiling of chromonemata or chromatids in all chromosomes of any one microspore mother cell, but it has been possible to get such data for one or several chromosomes in many different cells. An analysis of 165 rod bivalents showed the same direction of coiling on both sides of the terminal chiasma in 91 cases (fig. 1) and a reversal of coiling in 74 cases (fig. 7). The direction of coiling has never been observed to change between the spindle fiber attachment and the distal end of the chromosome, although it is probable that it does so at times where interstitial chiasmata are found. The direction of coiling of the chromonemata may change at the fiber attachment point. In the 165 bivalents studied there were 246 single chromosomes with the chromonemata coiled in the same direction for the entire length of each homologous chromosome, and only 84 chromosomes showed a reversal of coiling at the fiber attachment. Reversal of coiling of the chromonema is shown in the lower homologue in figure 2.

The direction of coiling of the chromatids at anaphase was also studied. Many of these chromosomes were doubtless associated as ring bivalents at metaphase, and the data obtained regarding the direction of coiling on either side of the fiber attachment are perhaps more nearly representative than data obtained from rod bivalents at metaphase. In all cases observed, the two associated (sister) chromatids coiled in the same direction at any given locus. In 90 anaphase chromosomes the direction of coiling was the same throughout the entire length of the chromosomes, and in 38 cases the

coiling was in opposite directions in the two arms of the chromosomes. Reversal of coiling at the fiber attachment can be observed in the anaphase chromosome shown in figure 11.

About 80 per cent of the bivalent chromosomes are associated by terminal chiasmata (or terminal affinity). Most of the subterminal chiasmata observed were so near the distal ends of the chromosomes that it was impossible to obtain data on the direction of coiling on either side of a chiasma. In several cases chiasmata were found near the spindle fiber attachment point, and in one of these bivalents the direction of coiling was reversed at the chiasma.

The pairing of homologous chromosomes by terminal or subterminal chiasmata at the first meiotic metaphase seldom involves the close association of chromatids. As a rule there is a clear space between the distal ends of the chromatids, as shown in figures 2, 3, 5, 8, and 14. This type of association is especially clear in the chromosome fragments. The distance between sister chromatids is about the same as the distance between the ends of non-sister chromatids, and in case of interstitial chiasmata in paired fragments a perfectly symmetrical double cross is often formed. For convenience in discussion, it is assumed that the first meiotic division is reductional, and that no crossing over has occurred.

In both aceto-carmine and permanent smears a hyaline area is often observed around the coiled chromonemata or coiled chromatids. Such a condition suggests that the chromosomes are inclosed by a limiting membrane or pellicle. In cells which have been crushed in smearing, this pellicle is often distended so that there may be considerable space between the cytoplasm and the coiled chromonemata. In such cases the two spindle fiber attachment points, one for each chromatid, are frequently found attached to the pellicle and connected with the coiled chromonema by two fine chromatic strands. These figures seem to show that the fiber attachment points are closely associated with, or definitely attached to, the chromosome pellicle or limiting membrane.

Cause and nature of chromosome coiling

Analysis of coiling and chromonemata and chromatids in *Tradescantia* confirms KUWADA's description, and is not in accord with

the interpretation of DARLINGTON and CATCHESIDE. The two chromatids are coiled together in a single chromonema in such a manner that the chromatids can separate without entangling. Such coiling could be induced by compressing the closely associated chromatids within the limiting pellicle in such a way that the chromatids are not permitted to rotate. This type of coiling can be simulated by compressing two closely associated flexible wires in a glass tube while the ends of the wires are not permitted to rotate. The chromatids are undoubtedly somewhat elastic and flexible, as shown by their behavior at division (SAX 9). They may be prevented from rotating within the pellicle by the association between the fiber attachment points and the pellicle. Inhibition of rotation at the distal ends of the chromosomes may also be affected to some extent by the terminal or subterminal chiasmata. The coiling produced in this way would produce the configurations observed. The chromatids, when coiled together at early metaphase, would appear to form a cross in certain chromonematic coils; the coiled chromatids can separate without entangling; and the sister chromatids have the same number of coils, the same direction of coiling at a given locus, and appear identical in any minor irregularities.

The coiling observed in the chromosomes at the second meiotic division is undoubtedly a secondary coiling which exists within the primary coils at the first meiotic division, as described by KUWADA (5). At the second division the primary coiling is absent and only the secondary coiling remains. The coiled chromatids at the first meiotic division are only about half as long as the chromosomes at the second division, but the actual length of the chromatid is about three times the length of the primary coil, so that there must be some contraction of the chromatid, between the first and second meiotic division, by an increase or contraction in secondary coiling. Such secondary coiling may account for the straightening out of the primary coil during the first meiotic division described in *Secale* (9), and chromosome contraction with no primary coils in the meiotic chromosomes of various *Orthoptera* species. BRIDGES' suggestion that coiling is a mechanism essential for the preservation of the linear order of the genes during contraction of the chromosome is in accord with cytological observations if secondary coiling is involved.

The direction of coiling of chromonema and chromatids seems to

be at random for the two homologous chromosomes of each bivalent. In the bivalents described by NEBEL (7) the direction of coiling was approximately at random for paired homologues. In our analysis of rod bivalents, 91 showed the same direction of coiling through the chiasma, and 74 showed a reversal of coiling at the chiasma. This means that when the chromosomes were paired side by side in the prophase stages, there was a slight excess of coiling in different directions, but the differences observed are of doubtful statistical significance.

The direction of coiling on either side of the spindle attachment in the meiotic homologues does not seem to be at random. NEBEL found 83 chromosomes with the direction of coiling unchanged in the two arms of the chromosomes, and 61 chromosomes with a reversal of coiling at the fiber. In our analysis of 128 anaphase chromosomes the direction of coiling of chromatids was the same in 90 chromosomes and was reversed at the fiber in 38 chromosomes. There seems to be a strong tendency for the coiling to be in the same direction at all loci in a meiotic chromosome. The reversal of coiling at the fiber might be expected if the fiber attachment point were a weak place in the chromatid. Such a point of weakness would break the continuity of stress imposed by compression or contraction of the chromatid.

In the case of interstitial chiasmata we should also expect more or less independence of coiling on either side of a chiasma.

NEBEL has observed anaphase chromosomes where the two chromatids on the same side of the fiber coiled in opposite directions, and we have observed one metaphase figure which would give such a segregation. NEBEL is inclined to interpret such figures as products of crossing over according to JANSSEN's theory. It is perfectly obvious that such configurations would be expected with either of the two current theories of chiasma formation.

The usual absence of any intimate association of homologous chromatids at the terminal and subterminal chiasmata seems to invalidate the theory of terminal affinity proposed by O'MARA (8) and by DARLINGTON (3). The homologous chromosomes seem to be associated by a union of the chromosome pellicles, and not by any unusual attraction or bond between the two terminal chromomeres.

According to NEBEL, each of the four coiled chromatids is split so that the bivalent chromosome at metaphase is really eight-parted. We have found no evidence that the chromatids are split, either at the first or second meiotic division. The anaphase chromosomes at the second meiotic division appear to contain a single coiled chromatic thread.

Summary

1. The chromatids of *Tradescantia* chromosomes are coiled together in a single coiled chromonema at early metaphase of the first meiotic division. At late metaphase the two coiled chromatids in each homologue separate, forming a bivalent with four separate coiled chromatids. The primary coils disappear during interphase, and at the second meiotic division only the secondary coils are found.

2. The primary coils of meiotic chromosomes are assumed to be caused by the contraction or compression of the two flexible chromatids within the chromosome pellicle without rotation of the chromatids.

3. The direction of the primary coils is apparently at random for the homologous chromosomes of each bivalent. Within each homologue the direction of coiling may change at the fiber constriction, but there is a tendency for the coiling to be in the same direction on both sides of the fiber. The direction of coiling may also change at an interstitial chiasma.

4. We have found no evidence that the meiotic chromatids are split in preparation for the division in the microspore.

ARNOLD ARBORETUM, HARVARD UNIVERSITY
DEPARTMENT OF BOTANY, IOWA STATE COLLEGE

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EXPLANATION OF PLATE IV

FIGS. 1–15.—Meiotic chromosomes from permanent smear preparations of microspore mother cells of *Tradescantia*. ×2000.

FIG. 1.—Rod bivalent with single coiled chromonemata.

FIG. 2.—Bivalent with subterminal chiasma. Direction of coiling of chromonema is reversed at fiber attachment of lower homologue.

FIG. 3.—Chromatids separating.

FIG. 4.—Separation of coiled chromatids in each homologue.

FIG. 5.—Rod bivalent showing nature of terminal association of homologous chromatids.

FIG. 6.—Early anaphase stage with single coiled chromonemata. Dual nature of chromonema can be seen in the upper homologue.

FIG. 7.—Partial opening out of coiled chromatids at anaphase.

FIGS. 8, 9.—Separation of coiled chromatids at metaphase.

FIGS. 10–12.—Anaphase chromosomes showing direction of coiling of sister chromatids.

FIG. 13.—Late anaphase chromosomes showing characteristic configurations of the six chromosomes.

FIG. 14.—Types of trivalents found in a triploid hybrid. Chromatids showing various degrees of separation.

FIG. 15.—Chromosomes of second meiotic division. Primary coils lost and each original chromatid shows secondary internal coiling.

SAX & HUMPHREY on TRADESCANTIA

SCHILDERIA ADAMANICA: A NEW FOSSIL WOOD FROM THE PETRIFIED FORESTS OF ARIZONA

L. H. DAUGHERTY

(WITH PLATE V)

During the fall of 1932, Mr. J. A. SCHILDER gave the writer a specimen of wood from the petrified forests of Arizona. Slides were made and it soon became evident that it represents a new wood from the Triassic, and a type of structure not previously reported either in living or fossil plants, justifying the establishment of a new genus. Upon the advice of Dr. I. W. BAILEY, who has examined the slides, it has been decided that, since the specimen shows only the secondary xylem, no suggestions may profitably be made concerning its relationship with other plants until evidence is obtained from additional material showing primary xylem, pith, or leaf traces.

The specimen has been replaced by chalcedony and its petrifaction is similar to that ordinarily found in the Arizona fossils. Figure 1 (pl. V) indicates the size of the specimen and shows the conspicuous multiseriate xylem rays.

Schilderia adamanica gen. et sp. nov.

TRANSVERSE SECTION (fig. 4).—Growth rings present, distinct, inconspicuous, occasionally doubled and averaging 1.5 mm. in width; variation in width from less than 0.4 mm. to more than 3 mm. Growth rings terminated by from one to five rows of flattened tracheids and a small amount of xylem parenchyma; cells of parenchyma solitary, in groups of two to four or occasionally forming a tangential row. In addition to the terminal parenchyma, some diffuse parenchyma can be found, but owing to the poor preservation it is rather difficult to demonstrate.

The large multiseriate xylem rays (medullary rays) form the most characteristic elements of the transverse section. These are of the "herring-bone" type found in the living genus *Ephedra* and in a few living genera of dicotyledons. They are 1–3 mm. apart, and two or three narrow uniseriate rays may usually be seen between them.

The tracheids appear large, squarish, heavy walled, many over 50 μ in tangential diameter, and with walls approximately 9 μ in thickness. It is possible, however, that the wall thickness has been slightly increased during petrifaction.

RADIAL SECTION (figs. 2, 3, 5, 6).—In this and the tangential section the tracheids prove to be of great length and show both pointed and blunt ends. The bordered pits on the radial walls are commonly localized in certain portions of the tracheid and are uniseriate or biseriate. The pits measure approximately 14 μ in height and 18 μ in width. In the uniseriate condition they are slightly flattened above and below by mutual pressure of the neighboring pits; in the biseriate condition their margins are angled, and the pits of the two rows alternate as is common in the pitting of the araucarian type (figs. 5, 6). The orifice of the pits ranges from almost circular to slitlike, and from a horizontal to an oblique position. When the oblique and slitlike orifice is present, it regularly forms a distinct cross with the orifice on the opposite side of the bordered pit. Bars resembling trabeculae are found extending across the lumina of the tracheids in a radial direction; it is possible that these may represent the hyphae of a member of the wood-destroying fungi.

The multiseriate rays (fig. 3) are composed of a combination of parenchyma cells and tracheids that bend into the ray and unite with it. The tracheids joining with the ray apparently undergo septation and form cells similiar to xylem ray tracheids, as the pitting remains similar to that found on the ordinary upright tracheids. The parenchyma ray cells appear to have either pitted or smooth walls where the preservation is good and the minute structure can be seen, but this fact cannot be established with certainty. Many of the details of the multiseriate ray cannot be worked out satisfactorily with the available material, but the fact remains that it is a composite structure, organized only partially from radial parenchyma and consisting largely of longitudinal tracheids that enter the ray.

The uniseriate rays are from one to twelve cells in height, composed of thin walled cells, and usually homogeneous. Heterogeneous rays with certain cells of the ray greater in height than in radial length may be found; the tall ray cells may be marginal, in the central portion of the ray, or make up the entire ray in case it is only

one or two cells in height. The cells of the central portion of the ray are approximately 30 μ in height and 140 μ in radial length. The lateral walls appear to have from two to six half-bordered pits in each crossing-field; at least some of the well preserved pits are half-bordered and the lateral walls show numerous circular openings believed to be remnants of pits.

TANGENTIAL SECTION (fig. 7).—The tracheids show tangential pits of two sizes, the larger approximately the same size as those on the radial walls, and the smaller about half that size. The pits are circular to oblong in shape and those examined had a slitlike orifice. Spirals may in some cases be seen on the walls of the tracheids, but these are probably the remnants of spiral striations rather than spiral thickenings.

The xylem parenchyma cells are 60–180 μ in length, the larger with simple pits on the end walls similar to those found in *Taxodium* and *Sequoia*.

The multiseriate rays show an approximate height of from 5 to 10 mm. and a width of 0.3 mm. The cells appear very irregular in shape in the tangential section, on account of their odd arrangement in the ray. The uniseriate rays are inconspicuous in the tangential sections examined. This is due to the fact that they are rather narrow in tangential width. The tangential width of those measured is approximately 10 μ.

It is evident from the description of **Schilderia adamanica** that its wood is of a different type from that of the other two fossil species from this locality. *Araucarioxylon arizonicum* was described by KNOWLTON (1) in 1888 and *Woodworthia arizonica* by JEFFREY (2) in 1910; since that time no new woods have been reported from the Triassic forests of Arizona. It is possible that a careful search of this locality will not only give valuable information in regard to the three species now known, but may result in the discovery of additional new elements of this flora.

The writer expresses his thanks to Dr. I. W. BAILEY for his helpful advice, and for the use of slides during the course of this work.

DEPARTMENT OF PALEONTOLOGY
UNIVERSITY OF CALIFORNIA
BERKELEY, CALIF.

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EXPLANATION OF PLATE V

FIG. 1.—Type specimen showing conspicuous multiseriate rays.
FIG. 2.—Radial section showing uniseriate ray; ×80.
FIG. 3.—Radial section showing multiseriate ray; ×80.
FIG. 4.—Transverse section showing multiseriate and uniseriate rays; ×80.
FIG. 5.—Radial section showing uniseriate type of pitting; ×80.
FIG. 6.—Radial section showing biseriate type of pitting; ×95.
FIG. 7.—Tangential section showing portion of a multiseriate ray; ×80.

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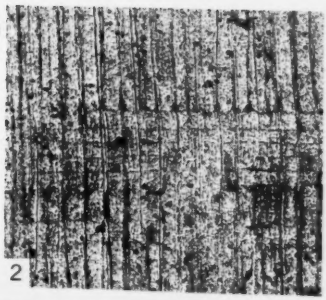

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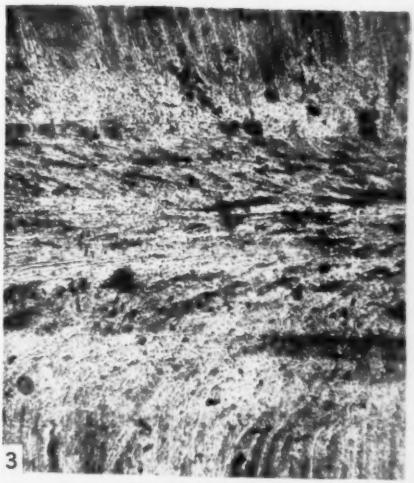

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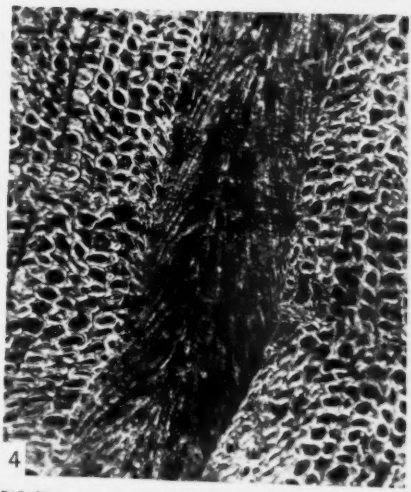

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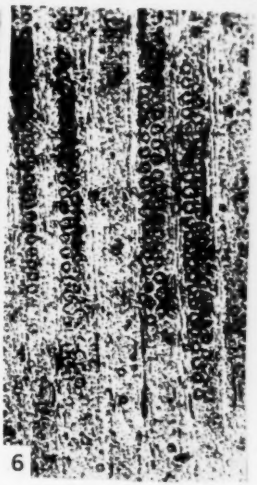

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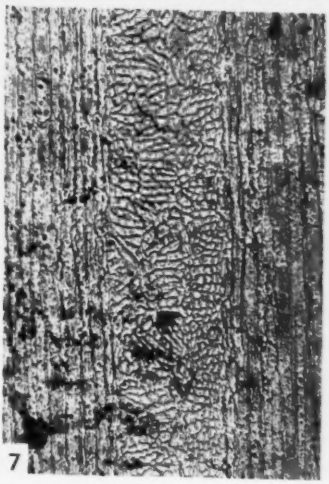


7

DAUGHERTY on NEW FOSSIL WOOD

ANATOMY OF AERIAL ROOTS OF VITIS ROTUNDIFOLIA

LEWIS M. TURNER

(WITH FIVE FIGURES)

Introduction

BAILEY (1), HEDRICK (4), and SMALL (6) have noted that aerial roots are often present on *Vitis rotundifolia*. They are not so long as, but they otherwise resemble, those of an unnamed, tropical conservatory species as described by MOORE (5). Whereas many die at the end of the first season, a few enter the soil and continue growth, the subterranean part resembling regular soil roots and the aerial part the canes, of course with the absence of nodes, shoots, and so forth. Lateral root development on the aerial part is common the first year, with two, three, or four such arising at a level.

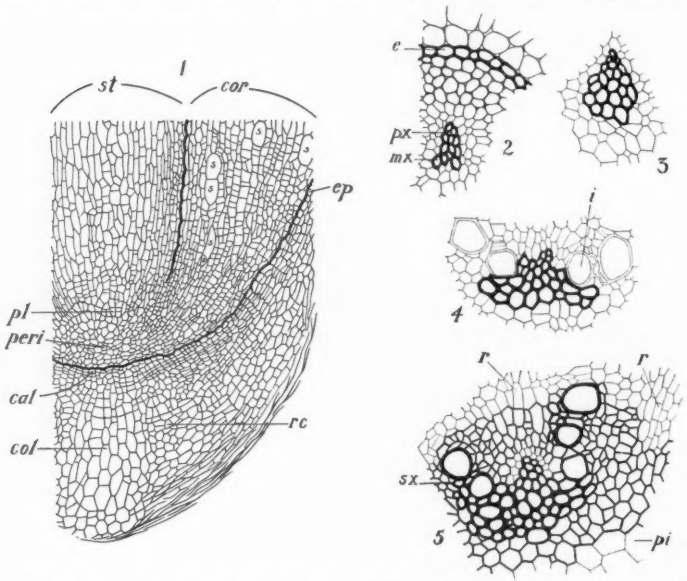
The roots originate on horizontal or oblique canes, usually on the lower side, and their point of origin may be at, near, or remote from the nodes. Since they arise most commonly on canes 1 inch or more in diameter, it seems reasonable to conclude that the phloem parenchyma is their histological source, the pericycle presumably having been lost through periderm formation by the phloem. Since, however, it was not found practical to make a thorough investigation of this problem, the foregoing conclusion is tentative.

Investigation

PRIMARY GROWTH

Three histogens, calyptrogen, periblem, and plerome, are distinguishable with difficulty in the apical region of the root. Resembling type II, of the various modes of root cap formation (2), and the root tip of the sweet potato (3), the calyptrogen divides early to form the epidermis, the latter persisting as an undivided layer throughout its existence. Except in one respect the performance of the meristems is normal, and is as follows: In the apical center of the conical root cap is a cylindrically shaped area of cells (fig. 1).

This is the "columella" described by NEMEC (HABERLANDT **2**) of supposed statolithic function. Its cells are cylindrically shaped with their long axes paralleling the long axis of the root and apposed to the long axes of the adjacent cells of the root cap. In conformity with the statolithic theory it was observed that these cells contain starch grains which are most commonly in the terminal position.



FIGS. 1–5.—Fig. 1, median longisection of apical region of root; figs. 2–5, progressive development of xylem, transverse sections (*cal*, calyptrogen; *col*, columella; *cor*, cortex; *e*, endodermis; *ep*, epidermis; *i*, immature secondary xylem; *mx*, metaxylem; *peri*, periblem; *pi*, pith; *pl*, plerome; *px*, protoxylem; *r*, ray; *rc*, root cap; *st*, stele; *sx*, secondary xylem).

The cortex is finally 15 to 25 cells in thickness. Its cells are elongated, with prominent intercellular spaces, and early two or more of its outer layers become collenchyma. This impervious layer would seemingly preclude the possibility of water absorption and in fact raise a serious doubt as to any absorption performance by the aerial part of these roots. The epidermis is lost very early, no stomata are formed, nor were the "pneumathodes" as of orchid roots (**2**) observed. Storage of crystals and stainable materials in the cortical

cells occurs early in the ontogeny of the root. "Crystal sacs" are visibly differentiated a short distance from the apex, large raphides are formed, are subsequently dissolved, and the cells that contained them collapse.

The endodermis is early distinguishable by the presence of stainable materials in the protoplasm of its cells. As well as could be determined typical Casparian bands are uncommonly found in the irregularly shaped endodermal cells. As a rule there occurs an uneven or interrupted secondary thickening of the walls of these cells. Storage is common, accumulation of materials taking place concurrently with wall thickening, so that by the time the cambium is differentiated the cells are opaque with only moderate staining. Accessory thickening of, and storage in, one or two adjacent cortical layers as occurs in *Taxus*, Cupressineae, *Viburnum*, and some Pomaceae (**2**) is commonly found.

The primary stele has six, seven, or eight protoxylem points. They are laterally separated by an equal number of primary phloem areas and fundamental parenchyma, and internally by a wide area or central core of pith parenchyma. The pericycle, distinguishable only by its proximity to the endodermis, is separated from the protoxylem points by four or five layers of parenchymatous cells (fig. 2). The centripetally developing protoxylem consists of six to eight spirally or annularly thickened elements. Subsequently several scalariform, and later a few reticulated metaxylem elements, with oblique end walls, develop centripetally and tangentially.

In general the direction of differentiation from primary to secondary xylem is at first centripetal, then tangential (from both corners of the protoxylem "triangle"), and finally centrifugal (figs. 2–5). A large pith core remains in the axis.

The first phloem elements are elongated parenchymatous cells with oblique end walls. Later elements, presumably metaphloem, are tubes of larger diameter with oblique end walls having small scattered, secondarily thickened areas.

Secondary growth

Cambium is differentiated in the usual manner and position, forming at first an undulating line (transverse view) between primary

phloem and xylem. Two or three layers of the fundamental parenchyma opposite and tangential to the protoxylem points remain undivided, and either become the first and innermost cells of the primary rays or remain parenchymatous. As has been suggested, immediately over the protoxylem points a ray is started. Tangential to this and external to the last formed metaxylem elements, the usual complex of secondary xylem is laid down. Simultaneously, at the point outside the gap between adjacent metaxylem areas is begun another type of ray, as seen in figure 5. There is nothing unusual in subsequent cambial activity, hence it will not be discussed except to describe the resulting tissues.

Secondary tissues

In transverse section the extra-cambial region is marked by conspicuous, elongated, triangularly shaped phloem areas. Alternating with these and opposite the xylem rays are inverted triangularly shaped areas of suberized cells, for the most part isodiametric but occasionally radially elongated. Scattered strands of fibers or single fibers extend more or less longitudinally through these areas. The sieve tubes, with oblique end walls and with elongate-oval sieve faces, are commonly 40–50 μ in diameter. Their radial walls have well developed lattices with small spots of secondary thickening scattered between the bars of the lattices. In roots up to seven years old there was little evidence of periderm formation; it is recognized, of course, that the canes of this species do not exhibit the same extensive periderm formation and shedding as do other species of *Vitis*. Small oval lenticels occur in longitudinal rows in young roots, but do not seem to attain the proportions and frequency of those of the aerial roots of the unnamed tropical grape (**5**).

The large vessels form the most conspicuous characteristic of the wood. Many, as in seven year old roots, were 200 μ or more in diameter. As would be expected these large tracheae are heavily walled, as much as 10 μ thick. The type of wall thickening is either scalariform or reticulated. Tyloses are fairly common. Fibers, fiber tracheids, and reticulate walled tracheids comprise the bulk of the remaining part of the wood. The meager wood parenchyma is vasicentric. Rays from one to ten cells in thickness and many cells in height are

made up solely of parenchyma. Annual radial growth of wood averaged approximately 1 mm. in the several roots studied.

The walls of the pith cells thicken slightly, but even in the seven year old roots the cells seem to be alive. This is evidenced by the presence of protoplasm and the abundant storage of materials.

College of Agriculture
University of Arkansas
Fayetteville, Arkansas

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EFFECT OF AFTER-RIPENING TREATMENT ON GERMINATION OF WHITE PINE SEEDS OF DIFFERENT AGES

HENRY I. BALDWIN

(WITH ONE FIGURE)

Introduction

It has long been a matter of common observation that seeds of white pine (*Pinus strobus* L.) germinate more promptly and completely if first subjected to moist storage at low temperatures, or even if incubated at lower temperatures than are customary in germination tests. Thus JACOBSEN (**7**) reported better germination outside on a veranda during the winter in Denmark than in the laboratory. BERRY (**2**) recommended cold storage and PETHERAM (**9**), fall sowing for white pine. Studies have shown that in a closely related species, western white pine (*P. monticola* D. Don.) (**11**), and in other western conifers (**4, 6**) fall sowing is definitely preferable to spring sowing. GRISCH and LAKON (**5**) have made a comprehensive study of after-ripening of white pine seed, and BARTON (**1**) reported 69 per cent in 24 days after two months' stratification at 5° C. compared with but 3 per cent in 60 days obtained from untreated controls. Similar results have been reported by the Lake States Forest Experiment Station (**12**) and by SCHMIDT (**10**). While fall sowing would seem to be the natural solution of the problem of securing good germination in nursery practice, and is the custom at a number of nurseries, CROCKER (**3**) recommends stratification for a month or two prior to spring sowing as preferable under some conditions. Since the completion of this paper, KOBLET (**8**) has reported the results of thorough tests of different temperatures and periods of storage. He found that the suddenness of change from one temperature to another had no influence on the course of germination. Stratification temperatures from 0° to 12° C. were about equally effective, but 9°–12° C. was better than 0°–3° C. The tests reported here differ from the other investigations cited chiefly in dealing with

a number of lots of known age, as well as three samples of unknown age and origin.

Methods

All germination tests were made on the Jacobsen germinator at a constant temperature of 24° C. Moisture supply, light, and acidity

TABLE I

AVERAGE GERMINATION OF WHITE PINE SEEDS OF DIFFERENT AGES WITH AND WITHOUT STRATIFICATION

Lot no.	Age of seed at time of testing (years)	Germinative energy in 30 days (percentage)						Percentage increase in germinative energy of stratified seed over dry seed	Stratification period (weeks)
		Dry untreated seed	Stratified in moist peat at 8°–10° C.						
			2 weeks	4 weeks	6 weeks	8 weeks	16 weeks		
1	5.5	2.0	6.5	11	.....	.....	.....	450	4
2	4.5	0	0	0	.....	0	.....	0	4
3	4.0	21.5	.....	36	12.5	.....	11	67.5	4
4	3.5	9.5	26.5	36	.....	.....	.....	300	4
5	3.0	30.5	.....	.....	.....	67	.....	120	8
6	3.0	21	.....	59	31	.....	59	181	4
7	2	30.5	.....	.....	.....	52	.....	70	8
8	2	14.5	.....	74	73	.....	86	410	4
9	2	20.5	.....	87	76	.....	94	310	4
10	1.5	12.5	51.5	72	.....	.....	.....	500	4
11	1	18.5	.....	.....	.....	92	.....	400	8
12	1	26.5	.....	.....	.....	90	.....	240	8
13	1	16.5	.....	57.5	47.5	.....	72	255	4
14	1	36.5	.....	85	85.5	.....	79	133	4
15	1	35	.....	97	89.5	.....	90	178	4
16	0.5	41	.....	77	.....	.....	.....	88	4
17	0.5	30	.....	13	.....	.....	.....	Decrease	4
18	0.5	19	.....	78	.....	.....	.....	310	4
19	0.2	67.5	.....	93	.....	.....	.....	38	4
20	0.2	59.5	74.5	86	.....	.....	.....	25	4
21	0.2	41.5	.....	.....	68.3	.....	.....	65	6
22	0.2	27.5	69.5	59	.....	.....	.....	119	4
23	? Probably over 3	3.5	.....	18	.....	.....	.....	415	4
24	? Probably over 3	11	.....	67	.....	.....	.....	510	4

remained constant. No disinfectants were employed. When mold developed the seeds were scrubbed in tap water and wicks and filter papers renewed. The seeds were stratified by mixing thoroughly

with granular peat in small glass jars. These were covered with pieces of cheesecloth held in place with elastic bands. The peat was

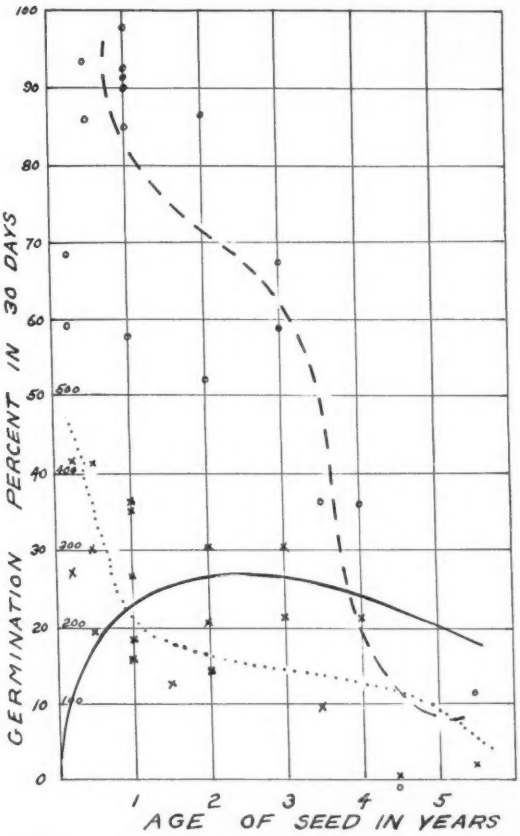

FIG. 1.—Comparison of after-ripened and dry seed, an increase of germination of after-ripened over that of dry seed. Unbroken line, percentage increase; dotted line, dry seed; dashed line, stratified seed.

moistened and stirred over at weekly intervals to insure aeration. The jars were stored in a refrigerator at 8°–10° C. from two to sixteen weeks, generally four weeks, before being transferred to the germinator. At least two samples of 100 seeds each were used in each test.

Results and discussion

Table I gives a condensed summary of the results. It is evident that germination was greatly hastened by after-ripening, except in two lots. One of these was probably non-viable seed; at least no germination ever occurred during 60-day tests. In a few cases better germination was secured with longer stratification, but generally four weeks was sufficient to induce very complete germination in a subsequent 30-day period.

A relation between age of seed and effectiveness of after-ripening is not very clear from the data. Apparently the greatest effect was produced in two- to three-year old seed (fig. 1). It might be supposed that the older the seed the more dormant it may have become, especially if stored in a warm place. The data are unsatisfactory in resulting from so many different lots of seed, originating from different seed crops. KOBLET found extreme variability in the behavior of white pine seed of different origin and seed crop, and it is likely that the present data were affected by like influences.

Summary

Germination tests were made of several lots of white pine (*Pinus strobus* L.) seed of different ages. In most cases after-ripening for several weeks at 8°–10° C. increased the rate of germination markedly. This increase was most prominent in the case of seed two to three years old. Germination decreased with age, that of stratified more rapidly than that of untreated control seed.

STATE OF NEW HAMPSHIRE
FORESTRY DEPARTMENT
HILLSBORO, NEW HAMPSHIRE

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CURRENT LITERATURE

Researches on fungi

The fifth volume of BULLER's now classic series[1] is divided into two parts, each distinct in subject matter. Part I, regarded by the author as a continuation of the latter part of the preceding volume, is concerned with the structure and function of the mycelium. Part II is concerned with spore discharge and homologies in *Sphaerobolus* and *Tilletia* (the latter in conjunction with T. C. VANTERPOOL), and with the mechanism of peridiole discharge in *Sphaerobolus*. The numerous illustrations are conveniently located with reference to the text, and are, with one or two exceptions, beautifully clear. A meaty summary, occupying 13 pages, and an exhaustive index, facilitate reference.

Hyphal fusions are shown to be of common occurrence, not only in the assimilative mycelium but also in the fructifications of the Ascomycetes and Basidiomycetes. They are held, in the former case, to facilitate the flow of nutrients, and in the fructification, to unify its structure and increase its mechanical stability. The various types of fusion are analyzed and it is shown that all fusions are between hyphal tips, either of undifferentiated hyphae or of peglike outgrowths. Clamp connections are included as one of the four recognized types of fusion and are regarded as being concerned wholly with growth and nutrition, their homology with the croziers of ascogenous hyphae being denied. In connection with the translocation of protoplasm, emphasis is laid upon the important but neglected work of WAHRLICH, and many of his illustrations are reproduced, showing protoplasmic connections between the cells of various fungi. The author describes in great detail the passage of cytoplasm, vacuoles, and nuclei through the pores, and suggests that the phenomena described are adequate to account for the rapid diploidization of haploid mycelium, once its complementary strain is introduced.

It has long been obvious that in the higher fungi large quantities of food must be transported from mycelium to hymenium during the brief period of fructification. BULLER's summary of neglected data and his wealth of additional observations go far to explain how this may be possible. His denial of sexual significance to the clamp connections will carry less conviction, more particularly as some of the facts concerning basidia and clamp connections upon which he bases his judgment, while true for the highly specialized agarics and similar forms, are far less applicable to the Heterobasidiomycetes and to

[1] BULLER, A. H. R., Researches on fungi. Vol. V. pp. xiii+416. figs. 174. Longmans, Green and Co., London. 1933.

the more simplified Homobasidiomycetes which must be regarded as transitional. Nor is adequate weight given, in the opinion of the reviewer, to the effect of the overwhelmingly increased importance of the diploid generation in Basidiomycetes as compared with Ascomycetes.

The studies on *Sporobolomyces* confirm the facts previously reported concerning this interesting yeastlike genus, and add to them certain particulars, notably the capacity of a single cell to produce up to three spores in succession on the same sterigma, or to produce three or four spores on separate or branched sterigmata. The author argues convincingly for the inclusion of the genus among the Basidiomycetes. Passing mention is made of BREFELD'S observation of similar behavior in the basidiospores of certain of the tremellaceous fungi, but this is not stressed, and recent studies showing that this phenomenon is widespread and common in that group are not mentioned. The habit of germination by repetition in moist air and of budding in yeastlike fashion in nutrient solutions, so characteristic of the basidiospores of the Tremellales, would seem to justify the conclusion not only that *Sporobolomyces* and *Bullera* are Basidiomycetes, but that they are imperfect members of the Tremellales.

A summary of the studies on *Tilletia* was published some years ago and is familiar to mycologists. As a result of their studies, it will be recalled, BULLER and VANTERPOOL concluded that the so-called spores of *Tilletia* are in reality greatly modified sterigmata, and the so-called conidia, primary and secondary basidiospores. The evidence for this point of view is given in great detail. But here again, adequate recognition of the functions and variations of the epibasidium and of the phenomenon of germination by repetition in the Heterobasidiomycetes might make the homology even more convincing.

The last chapter is devoted to a study of *Sphaerobolus*, including its range, kinetics, and ecological relationships. The genus is regarded as primarily coprophilous and only secondarily xylophilous. In view of the great abundance of *Sphaerobolus* on wood in localities to which herbivorous animals have no access, this point of view seems overstressed.

As is the case with previous volumes, the wealth of information it contains and the painstaking and exact attention to details make this a reference work which all mycologists must not merely read, but must keep within reach for constant reference. Its very importance, however, imposes critical appraisal of the conclusions which it presents.—G. W. MARTIN.

Carotenoids

Until a few years ago the chemical constitution of the carotenoids was essentially unknown. While empirical formulae for carotene and xanthophyll had been determined as $C_{40}H_{56}$ and $C_{40}H_{56}O_2$ respectively, these formulae gave no indication of the structural pattern of the molecules. During the last few years great progress has been made, and we can now consider the structural formulae of the carotenoids as fairly well established. In addition, the long suspected relationship between the yellow pigments and vitamin A has been

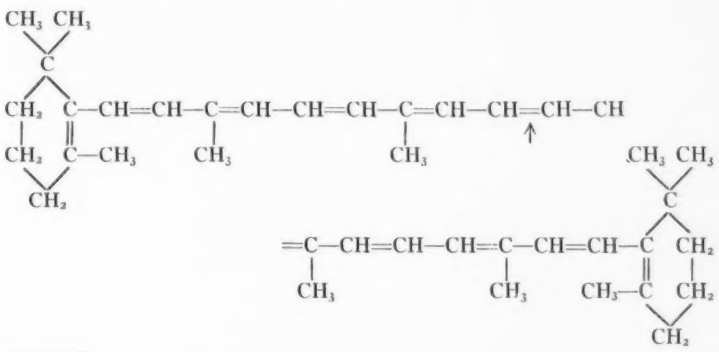
cleared up. It has been shown that vitamin A represents half of the molecule of B carotene, in the form of a polyene alcohol.

A very interesting monograph on the carotenoids has been prepared by ZECHMEISTER,[2] one of the ardent investigators in this field. It should be read by all plant physiologists, for it clarifies the entire field and puts the chemistry of carotenoids on as firm a basis as the chemistry of the chlorophylls, or the fats or sugars.

The main part of the monograph deals with the plant carotenoids, of which something like twenty are now known. The second section, dealing with the animal carotenoids, is very brief, and mainly devoted to the crustacean pigment astacin. The section on plant carotenoids is divided into two parts, a general part and a special part. The former discusses the general features of the carotenoids; the history of our knowledge of the yellow pigments; problems of structure and color; stereochemical relations; the biochemical construction of the pigments; the physiology and rôle of the pigments; their relations to other classes of chemical compounds such as the terpenes, sterols, vitamin A, lipoids, proteins, etc.; the methods of investigating the constitution of the carotenes; the occurrence and condition in the plant; and methods of identification, separation, and determination of the carotenoids.

The special part describes the specific carotenoids: first the 40-carbon polyene hydrocarbons, carotene and lycopene; and then the oxygen-containing pigments of the C-40 series, kryptoxanthin, rubixanthin, xanthophyll, lutein, helenien, zeaxanthin, physalien, flavoxanthin, violaxanthin, taraxanthin, fucoxanthin, rhodoxanthin, capsanthin, and capsorubin. There are four pigments with more or less than C-40: bixin ($C_{25}H_{30}O_4$), crocetin ($C_{24}H_{24}O_4$), crocin ($C_{44}H_{64}O_{24}$), and azafrin ($C_{27}H_{38}O_4$).

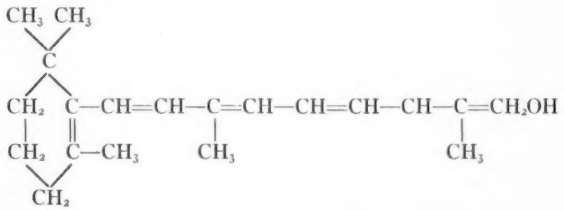
The formula for B carotene ($C_{40}H_{56}$) is as follows:



[2] ZECHMEISTER, L., Carotenoide. 8vo. pp. xii+338. figs. 85. Springer, Berlin. 1934.

The arrow indicates the center of the molecule, the two halves of the molecule being arranged symmetrically on opposite sides of the double bond.

Vitamin A is produced by the symmetrical breaking of the molecule at this center double bond, with the absorption of water to form a primary alcohol. The molecule of vitamin A is



The final section of the monograph contains 50 beautiful photomicrographs of the various crystallized carotenoids. A bibliography of 28 pages emphasizes the recent growth of carotenoid literature, for most of the papers have come out within the last five years.—C. A. SHULL.

Electrokinetic phenomena

Since the discovery of electroosmosis by REUSS in 1808, many observations have been made on biological materials which involve the electrical forces which reside on the surfaces of these colloidal substances. An important monograph on electrokinetic phenomena and their application to biological phenomena has been prepared by ABRAMSON[3] and published as monograph no. 66 of the American Chemical Society Monograph series.

The first chapter is a valuable historical summary of the discoveries during the period from the time of REUSS's work in 1808 to the formulation of the theory of electrical double layers by HELMHOLTZ in 1879. This is followed by a presentation of the HELMHOLTZ theory and its application to various phenomena such as electrophoresis, streaming potentials, viscous flow, sedimentation potentials, etc., and the early experiments which seemed to offer sufficient confirmation of the HELMHOLTZ hypothesis.

The third chapter considers the methods now in use in such experimental work. The use of ultramicroscopes, the problems of illumination, magnification, rotating and alternating fields, gas bubbles and liquid droplets, moving boundaries, streaming potentials in capillaries and through diaphragms, and methods of studying electroosmosis and sedimentation are discussed in illuminating manner. The fourth chapter considers the more recent theories such as those

[3] ABRAMSON, H. A., Electrokinetic phenomena and their application to biology and medicine. 8vo. pp. 331. figs. 106. Chemical Catalog Co., New York. 1934. $7.50.

of GOUY, and of DEBYE and HÜCKEL. The succeeding chapters concern specific biological systems and general surface phenomena. The proteins and related compounds such as hemoglobin, pepsin, etc., are taken up first, followed by consideration of salt effects on inert surfaces and inorganic surfaces in general. Glass, quartz, silica gel, clays, metallic colloids, metallic oxides, sulphides, carbon particles, etc. claim attention in this part of the work. Then the organic surfaces are considered, such as organic emulsoids, paraffin oils, phenol, guaiacol, benzonitrile, and aniline in aqueous media, lipide emulsions of many kinds, cellulose, amylose, glycogen, etc. Gases in colloidal form are discussed in a brief chapter; and finally such biological systems as blood cells, spermatozoa, frog skin, human skin, tooth enamel, trout eggs, onion skin, bacterial cells, anti-bodies, viruses, and related systems receive careful treatment.

Appendix I gives a list of symbols used in the mathematical discussion of potentials, and II contains a list of constants and conversion factors which are necessary in applying the formulae to specific problems. Appendix III refers to patents which have been granted on processes involving electrokinetics, such as drying of peat, electrodeposition of latex, purification of clay, water, sugar solutions, serum, and gases, separation of water and oil emulsions, and the impregnation and tanning of hides in leather manufacture.

The presentation is technical enough so that the reader must bring some knowledge of the field to the task of reading it. Nevertheless the reviewer feels that ABRAMSON has performed a real service to biologists in bringing these important and difficult problems into the compass of a relatively brief monograph. In masterly fashion he has drawn into a well connected presentation material from widely separated fields of physics, chemistry, and biology. Much of the material would remain obscure and inaccessible to biologists if not brought together by someone who understands the relationships involved. Electrokinetic phenomena are of great significance in biology, and this book will help to focus attention upon problems which have been overlooked and neglected in the past. Many investigators in the biological field will find the information in this monograph extremely useful.—C. A. SHULL.

Families of monocotyledons

In the review[4] of the first volume of this work,[5] it was predicted that it would be more widely noted and would stimulate more interest in the possible relationships among families of angiosperms than have many of the systems of classification which have appeared from time to time. The fulfilment of this

[4] BOT. GAZ. **82**: 111–112. 1926.

[5] HUTCHINSON, J., The families of flowering plants. Vol. II. Monocotyledons. Arranged according to a new system based on their probable phylogeny. 8vo. pp. xiv+243. figs. 107. Macmillan & Co., London. 1934. $6.

prophecy in the United States is shown in the use of HUTCHINSON's classification of the dicotyledons in taxonomy courses in several colleges. Many botanists have been awaiting with keen anticipation the appearance of this companion volume on the monocotyledons.

The author's preface states that "the principal object of the book is to provide the student with descriptions of the families of monocotyledons arranged in as logical a sequence as may be possible according to their probable phylogeny," and that "although somewhat drastic alterations are proposed, it should be understood that the work is not monographic, but represents only the beginning of an endeavour to establish a phylogenetic system for the monocotyledons." Nevertheless, HUTCHINSON has been able, during the eight years in which much of his attention has been concentrated on the study of the living and dried specimens at Kew, to build up a truly thought-provoking system. His treatment departs from that of BENTHAM and HOOKER even more than was true of his system of classification for the dicotyledons.

The group is considered to be monophyletic, showing close relationship to the dicotyledons only through the Butomales and Alismatales. These share with the Ranales an apocarpous gynoecium and a tendency to possess numerous stamens. Endosperm, which is regarded as a primitive feature in general, has been lost in these lower forms, probably owing to the adoption of an aquatic habit. These primitive stocks are placed with, and are considered to have given rise to, all the forms included under the first general division of the Calyciferae (calyx-bearers). These comprise, among others, the Commelinales, Bromeliales, Zingiberales, Xyridales, Eriocaulales, Potamogetonales, and Najadales. These are mostly forms of moist or aquatic habitat which either retain a biseriate perianth or show marked perianth reduction and sexual differentiation.

Somewhere from these primitive stocks, possibly from forms resembling *Scheuchzeria*, there arose a more terrestrial race, including such forms as the more primitive of the existing Liliaceae. This underwent prolific evolution to give rise to the remainder of the monocotyledons. Many of these, including the Liliales, Palmales, Burmanniales, Orchidales, Arales, Typhales, etc., are grouped by HUTCHINSON into a second great division, the Corolliferae (corolla-bearers).

A third climax group, the Glumiflorae, is thought to have branched off independently from the Liliaceous stock. It includes the Juncales, which in turn have given rise to the Cyperales and Graminales.

Conservative botanists will be startled at HUTCHINSON's treatment of the Liliaceous complex, especially the old groups of Liliaceae and Amaryllidaceae. Not only is his tendency to the reduction in size of families carried into effect with the recognition of the Trilliaceae, Smilacaceae, Ruscaceae, Agavaceae, Hypoxidaceae, and others, but he regards the type of inflorescence as of greater importance than epigyny or hypogyny. This results in a transfer of the tribes Agapantheae, Allieae, and Gilliesieae from the Liliaceae to the Amaryllidaceae. The latter thus becomes, according to HUTCHINSON, "a very homogeneous and

natural group, the most distinctive and constant feature of which is the umbellate, scapose inflorescence." The family as thus constituted is given ordinal rank as well, as are several other single families.

The orders and families recognized and described in this volume total 29 and 68 respectively, bringing HUTCHINSON'S total for the angiosperms up to 105 and 332.

The usefulness of the book is much increased by the inclusion of keys to the genera of all families except the Orchidaceae and Gramineae, in addition to a well planned artificial key to the families. Other desirable features, which were found in the preceding volume as well, are the numerous drawings, many of them original, of species which exhibit points of special phylogenetic interest. Sketch maps, showing the distribution of certain families, are also included.

HUTCHINSON acknowledges the assistance of Messrs. DANDY, HUBBARD, and SUMMERHAYES in the treatment of the Hydrocharitaceae, Gramineae, and Orchidaceae respectively. He most appropriately dedicates this volume to Dr. AGNES ARBER.—C. E. OLMSTED.

Gramineae

In the publication of this book[6] ARBER has brought together in one volume the results of many years of research devoted to the study of the monocotyledons, and of the grasses in particular. Admittedly no attempt has been made to include within the pages of one book all the facts and theories regarding such a large plant group. Instead, the author has limited herself to "those aspects of the subject which happen to make the greatest appeal. . . . . " Thus emphasis naturally falls upon the morphological treatment of the grasses, to which subject the author has devoted the major portion of her research. Humanistic, historical, and phylogenetic relationships receive a rather extended treatment while references to ecological, physiological, and taxonomic problems are more or less incidental.

The initial chapters are devoted to the humanistic, historical, and geographical phases of the grasses. In these, man's discovery and use of the grasses and their relation to the history of mankind are recounted. The cereal grasses, those used for pasture, sugar, and scent are considered in order.

Following this section are four chapters which treat the bamboos with respect to their vegetative phase, tree habit, reproductive phase, and spikelet and fruit. In discussing the tree habit, ARBER takes exception to the theory of the arboreal ancestry of the angiosperms, and suggests that this habit may be regarded as "an expression of racial senile degeneration . . . . as the final expression of a certain fundamental tendency in plant life." This she explains may be due to the development of the cellulose wall and the consequent loss of plasticity; and she concludes her argument with the question, "May we not then visualize the

[6] ARBER, AGNES, The Gramineae: A study of cereal, bamboo, and grass. pp. xvii+480. figs. 212. University Press, Cambridge and Macmillan Co., New York. 1934. $8.50.

tree habit as the ultimate expression of the liability to the accumulation of inert organic matter—a tendency which can be kept within bounds in the youthful phases of a race, but which is apt to pass out of control when senescence is reached?" In connection with the reproductive phases of bamboo, there is an interesting discussion of the phenomenon of gregarious blooming and the causes of periodic flowering.

The life cycle, reproductive and vegetative phases, embryology and morphology of the grasses are the subjects to which the major portion of the volume is devoted. The results of the author's extensive research are brought together and to some degree correlated with the work of other investigators. These chapters, as well as all others, are liberally foot-noted and there is an accompanying bibliography which includes most of the important contributions on the Gramineae.

The interpretation of such structures as the coleoptile, mesocotyl, and epiblast will doubtless provoke some controversy, for it is to be expected that workers in such a large plant group as this will be at variance in respect to many of the morphological conclusions presented. But there is a refreshing and stimulating unorthodoxy in the treatment of some of our formal morphological concepts, such as the impossibility of a leaf being terminal to a stem, the use of the term adventitious, and the question of root and shoot terminology. In this latter connection, the author concludes that "stem and leaf cannot be accepted as valid morphological categories."

The final chapters deal with problems of the distribution and dispersal, hybridization, and the pattern and rhythm of the grasses.

The declared purpose of this volume was "to detect the pattern and rhythm underlying that complex of plant types called the Gramineae." To the solution of this problem ARBER has contributed valuable data and critical thought; yet, in a sense, she confesses partial failure, for this excellent treatise concludes with the question and statement: "What is the *meaning* of the differences that separate the Gramineae so delicately, yet so definitely, from any other order, and that so prevail that a grass remains a grass, however freely that type may vary? . . . . The mystery abides."

But though the controversial aspects of grass anatomy and phylogeny may remain unsettled, there is no question but that this volume is an important and distinguished contribution to botanical literature, a treatise that will be generally recognized as a standard in its field. It is well illustrated, clearly written, and will undoubtedly be received with appreciation by all botanists interested in this phase of plant science.—H. E. HAYWARD.

Identification of commercial timbers

A new volume in the American Forestry series[7] devoted to the identification of commercial timbers of the United States has recently been published. This

[7] BROWN, H. P., and PANSHIN, A. J., Identification of the commercial timbers of the United States. pp. xxvi+223. Illustrated. McGraw-Hill Book Co., New York. 1934.

will be a welcome addition to the literature relating to those phases of forestry that deal with the anatomy and identification of our native commercial woods. The book is specifically designed to meet the practical needs of the college student, the forester, and the workers in industries dealing in wood products.

The organization of the book is logical, and consists of three major divisions. The first deals with the anatomy of woods and includes a discussion of the general concepts of tree growth, the physical properties of wood, the gross anatomical features of wood, and a concluding section on minute anatomy. While this section of the text is somewhat more brief than might be desired, it should afford sufficient information to enable the worker to use the keys for identification successfully. It is supplemented by a comprehensive glossary which includes all the terms referred to in the keys and descriptive portions of the text.

The second section of the volume consists of two keys for the identification of the commercial timbers of the United States. The first key is based on characters that can be discerned with the naked eye or with the aid of a hand lens (10×). The second key is based upon minute characters which require the use of a compound microscope. Accompanying the first key are photomicrographs of the cross sections of the woods (5×) while the key based on the microscopic characters is illustrated with cross and tangential sections (75×). The nomenclature used in the keys includes the common trade names as well as the scientific names.

The final section is devoted to descriptions of the species referred to in the keys. Each species is described in terms of its general characteristics and its minute anatomy. For the convenience of the reader, the plate numbers of each species are noted in connection with the description.

The uniformity of the plates and the general excellence of their reproduction together with the inclusion of tangential sections in each case add greatly to the value of the work.—H. E. HAYWARD.

Economic plants

A volume[8] has just appeared which provides the teacher of botany with a long needed work on the economic uses of plants. The increasing tendency to correlate the teaching of pure plant science with the applied and with the activities of everyday life has revealed a dearth of adequate material suitable for textual and collateral reading. The present volume is well designed to meet this need, and should also prove valuable as a source book for teachers in the high schools and the upper grades.

The volume is well indexed and the organization is logical. The introductory chapters deal with "the plant kingdom" and "cells, tissues, and organs." These are designed to orient the reader with respect to the major groups in

[8] STANFORD, E. E., Economic plants. pp. xxiii+571. figs. 376. Appleton-Century Co., New York. 1934. $4.50.

plant classification, plant nomenclature, and to provide the fundamental concepts and terminology of plant anatomy as well as the functions of the major plant organs.

The discussion of the economics of plants is divided into five major sections: (1) forest products, under which are chapters dealing with the composition and structure of wood; uses of wood; resins, tanning, and cork; rubber and latex products; (2) textile plants; (3) paper and pulp; (4) food products including cereal crops, sugar plants, fixed oils (not all food products), nitrogen products, fruits, spices and beverage plants; (5) medicinal plants.

The treatment of the subject matter is in part historical and geographical as well as economic. It is written in an interesting and readable style and is generously illustrated.

The scope of the book is so great that the discussion of many of the topics is necessarily brief. For this reason it is to be regretted that the author did not include lists for supplementary reading, either in connection with each chapter or as an appendix to the volume as a whole. This would have given added value to a work which in other respects is a worthwhile contribution to current botanical literature.—H. E. HAYWARD.

Wood identification

The value of RECORD's books on wood structure has been proved by the exhaustion of two editions of his earlier book, Economic woods of the United States, a notice of which appeared in this journal.[9]

A larger volume[10] has now appeared which is much more than a third edition. The first half of the book is entirely rewritten and much material has been added to the second part. It seems safe to say that the many who have found the earlier editions so useful will be even better pleased with the present book.

There has always been more or less confusion in the use of terms used to describe various details of wood structure. It is therefore gratifying to find the book using the terms recommended by the International Association of Wood Anatomists.[11] These terms have been defined in somewhat more detail than in the original report, and have received a practical application in the descriptions. The number of illustrations has been increased and many good photomicrographs have been included. One of the notable features of the book is an artificial key for the identification of 80 economic woods of the United States and Canada. Structures visible to the naked eye and those revealed by the use of a hand lens and by a compound microscope are the criteria for the identifica-

[9] BOT. GAZ. **68**:480. 1919.

[10] RECORD, S. J., Identification of the timbers of temperate North America. 8vo. pp. 196. pls. 6; figs. 47. John Wiley & Sons, New York. 1934. $3.

[11] COMMITTEE ON NOMENCLATURE: International Assn. of Wood Anatomists. Glossary of terms used in describing woods. Tropical Woods no. 36. 1–12. 1933.

tions. Experience has shown this key to be reliable but to require a very considerable background of experience on the part of the operator.—G. D. FULLER.

Raunkiaer's life forms

Perhaps the most important book of the year for plant ecologists and phytogeographers is the recent volume containing the collected papers of RAUNKIAER, translated into the English language.[12] The first of this series of contributions appeared over 30 years ago (1904), and like most of this author's papers, was in the Danish language. It was a preliminary statement of a classification of plants based on their adaptations to survive the unfavorable season. This arrangement gave a series of groups that soon became known as "Raunkiaer's life forms." This concept, together with its application in the form of "biological spectra," used to characterize the phytogeographical climates of the earth, has been RAUNKIAER's most notable contribution to plant ecology. It was promptly accepted by European ecologists and has become familiar to American investigators through translations into English of portions of RAUNKIAER's writings and through a later paper in German by RAUNKIAER himself.

Scarcely less notable than RAUNKIAER's life forms has been his contribution to the statistical study of plant communities. His methods of determining the degrees of frequency (valency) of the various members of the communities, now so widely used, were described in detail in a Danish paper which appeared in 1909 and in a French article dated 1918. Probably RAUNKIAER's contributions have done more to promote quantitative investigations in plant ecology than have those of any other ecologist. In this respect he has proved a worthy follower of WARMING.

The third notable contribution of this versatile scientist is his studies of the vegetation of the Mediterranean region which occupy not less than 100 pages of the present volume, including an article of 75 pages hitherto unpublished.

The translation of the Danish articles by H. GILBERT-CARTER of Cambridge, and Miss A. FANSBØLL has been well done, as has that of the German and French papers by A. G. TANSLEY, who has edited the whole volume. The Danish scientists in charge of the work were K. GRAM, H. MOLHOLM HANSEN, OVE PAULSEN, JOH. GRØNTVED, and the late C. H. OSTENFELD. To all of these English speaking ecologists we are indebted for a monumental volume which is certain to make the notable contributions of RAUNKIAER more widely and fully appreciated.—G. D. FULLER.

Flora of Buffalo and vicinity

Residents of Buffalo and vicinity and students of plant distribution will be interested in the present volume,[13] which is a floristic study of the vascular

[12] RAUNKIAER, C., The life forms of plants and statistical plant geography, being the collected papers of C. RAUNKIAER. pp. xvi+632. figs. 189; portrait. Clarendon Press, Oxford. 1934. $14.

[13] ZENKERT, C. A., The flora of the Niagara frontier region. Ferns and flowering plants of Buffalo, N. Y., and vicinity. Bull. Buffalo Soc. Nat. Sci. vol. XVI. pp. x+328. Illustrated. Buffalo Museum of Science, Buffalo, New York. 1934. $2.

plants found in the area within a radius of about 50 miles of Buffalo. The body of the work falls into four main sections: an historical account of plant exploration in the area; a discussion of the regional environmental conditions; an annotated list of the 1187 native and 400 introduced species of the region; and a description of the principal ecological areas and plant societies. The work is copiously illustrated.—C. E. OLMSTED.

Tropical and Japanese plant observations

In a recently published memoir[14] a great many references are made to a visit to Buitenzorg in 1898 and another visit to India in 1928–29, which the author of the memoir spent at the Institute for Plant Physiology of Sir JAGADI CHANDRA BOSE. The main researches of his first trip were on the formation of indigo, of palm wine, and on the secretion of water by liana stems. These studies were continued and enlarged during MOLISCH'S second trip to the tropics, and can be found discussed in his book, A Naturalist in India (1930).

Many other observations along the line of plant physiology were made during his prolonged stay in Japan, from 1922 to 1925, and are gathered in his book, Plant Physiology in Japan on the Basis of Personal Observations (1926).

MOLISCH writes in German but his diction is clear and concise and can easily be read by English speaking students who have some knowledge of German. —A. C. NOÉ.

Haemolytic systems

Monograph no. 6 of the Protoplasma Monographien series is a volume by PONDER[15] on red blood cells and haemolytic phenomena. There are ten chapters, dealing with counting methods, dimensions, shape and structure, and chemical composition and metabolism; permeability and osmotic haemolysis; properties of simple haemolytic systems; inhibition and acceleration of haemolysis; resistance series; systems containing sensitizing agents; and miscellaneous forms of haemolysis. There are several appendices, on sedimentation, rouleaux formation, stromatolysis, and the erythrocytes of the Camelidae. An extensive bibliography and subject and author indices are included.—C. A. SHULL.

Copies of the photograph of Professor H. C. COWLES, which appeared as a frontispiece in the September issue, may be secured by addressing the Editorial Office of the *Botanical Gazette*, University of Chicago, and inclosing stamps to cover cost and mailing. One copy, 18¢; each additional copy, 10¢.

[14] MOLISCH, HANS, Erinnerungen und Welteindrücke eines Naturforschers. pp. 232. Emil Haim, Wien and Leipzig. 1934. RM 9.

[15] PONDER, ERIC, The mammalian red cell and the properties of haemolytic systems. 8vo. pp. xii+311. figs. 52. Gebrüder Borntraeger, Berlin, 1934. RM 22.5.